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ScienceDirect
Bioorganic Chemistry 35 (2007) 258–275
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

New oligonucleotide analogues based on morpholine subunits joined by oxalyl diamide tether

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Received 24 October 2006
Available online 15 February 2007

Abstract

We report on the design, synthesis and some of the properties of the new oligonucleotide analogues based on morpholine nucleoside (MorB) subunits joined by an oxalyl diamide tether instead of a phosphate group. The synthetic strategy and oligomer design are optimized to easily obtain target substances without using protective groups. The dimers HOMorU-Ox-NHMorU, HOMorU-Ox-NHMorA, and uracil containing the hexamer HOMorU-(Ox-NHMorU)$_5$ were synthesized. The structures of all substances were confirmed by ^{1}H, ^{13}C, NMR, and mass spectroscopy. Base stacking interactions in dimers were revealed by CD-spectra data.

Keywords: Oligonucleotide analogues; Morpholine nucleosides; Aminonucleosides

1. Introduction

Even after several decades, interest in the construction and synthesis of a variety of novel oligonucleotide analogues continues. The first analogues—polyvinyl and polyacrylate based polymers—were studied in the early 1970s [1,2]. More sophisticated constructions,

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0045-2068/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2006.12.003

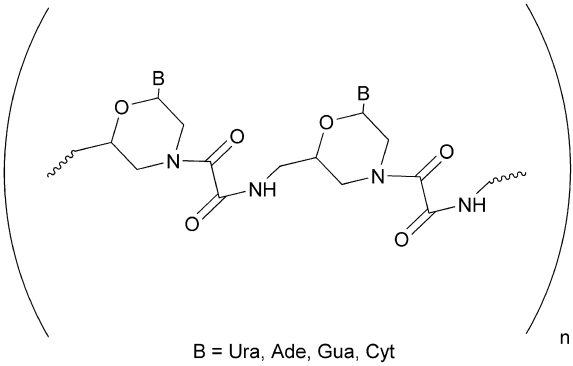


Fig. 1. The structure of morpholino oligonucleotide analogues with an oxalyl diamide backbone.

with a regular predetermined structure containing nucleoside units joined with carbonate, carbamate, hydroxyacetate and hydroxyacetamide tethers, were reviewed a little later [3]. The progress in oligonucleotide synthetic methods during the 1980s gave the impetus for the development of a variety of oligonucleotide analogues with modified carbohydrate and phosphate backbones. The achievements in this field up to early 1990s have been summarized in a monograph edited by S.T. Crooke and B. Lebleu [4]. In recent reviews [5–7], particular attention has been given to the PNA (peptide nucleic acids), Morpholine, and Negatively Charged PNA oligonucleotide analogues, which seemed most promising in a number of biological applications, such as diagnostics, nucleic acids analysis, and gene expression.

Nevertheless, chemists continue to put forth effort in design and synthesis of new substances mimicking nucleosides and oligonucleotides [8–14]. In some cases, authors initially synthesize only thymine containing substances [12,15–17], obviously due to the considerable synthetic challenges in the case of other heterocyclic bases.

Evidently, the cost of parent compounds and oligonucleotide analogue synthesis in general is one of the most important problems. The studies that succeed in resolving this problem in the best way would benefit from a practical point of view. Here we present the oligonucleotide analogues (Fig. 1) that can be synthesized starting from inexpensive parent compounds—ribonucleosides—without the protection of heterocyclic bases, and with minimal protection from other reactive functions.

2. Materials and methods

2.1. General

The following reagents and equipment were used: uridine, adenosine, guanosine, cytidine (ChemGenes Corporation, Wilmington, MA, USA), $NaJO_4$ (Fluka Chemie AG, Buchs, Switzerland). Oligodeoxynucleotides $d(C_2A_6C_2)$ and $(T)_6$ were purchased from Biosset (Novosibirsk, Russia). Monomers **1(a–d)**, **10** and **14** were synthesized according to published procedures [18,19]. *p*-Nitrophenyl chlorosulfate was synthesized following the previously-reported method [8]. All other reagents were from Aldrich (Milwaukee, WI, USA). Organic solvents were dried and purified by standard procedures.

NMR spectra were acquired on Bruker AM-400 and AV-300 instruments (Bruker, Bremen, Germany) in appropriate deuterated solvents at 30 °C. Chemical shifts (δ) are reported in ppm relative to TMS signals. Coupling constants *J* are reported in Herz. MALDI-TOF mass spectra were run on Reflex III (Bruker Daltonics, Bremen, Germany) in positive detector mode with dihydroxybenzoic acid as a matrix. IR spectra were recorded on a Vector 22 (Bruker Optics, Bremen, Germany) in KBr.

Analytical reverse phase HPLC was performed on a Milichrom-4 (Econova, Novosibirsk, Russia). A 2 × 60 mm column packed with Nucleosil C_{18} (5 μm), (Macherey-Nagel, Duren, Germany) having a gradient elution of acetonitrile in 0.05 M TEA-AcOH (0–50%) over 25 min, with an elution rate 0.1 mL per min, was used for the analysis. Semi-preparative reverse phase HPLC was performed using the Bruker ChromStar Chromatography system (Bruker, Bremen, Germany) and a 1 × 25 cm column packed with LiChroprep C18 (15–25 μm) (Merck, Darmstadt, Germany) with a linear gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid (TFA). The compositions of all liquid mixtures were indicated as v/v.

Preparative silica gel chromatography was performed on Kieselgel 55–100 μm (Merck, Darmstadt, Germany) using the appropriate eluents. Thin layer chromatography was performed using Alufolien Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany) in the appropriate solvent mixtures and visualised by UV irradiation, ninhydrin (amine groups) or cystein/aqueous sulfuric acid (nucleoside). All evaporations were performed under reduced pressure.

2.2. Monomer and oligomer synthesis

2.2.1. 2′-Aminomethyl-6′-(guanine-9-yl)-morpholine **8** (Scheme 1)

Monomer **1 d** (0.580 g, 1 mmol) and Ph_3P (1.5 mmol, 0.39 g) were dissolved in DMF (5 mL). Sodium azide (10 mmol, 0.65 g) and bromotrichloromethane (1.5 mmol, 0.15 mL) were then added to the reaction mixture with stirring. After stirring for 16 h the reaction mixture was evaporated, and the residue was distributed between 50 mL of dichloromethane containing 0.1% of pyridine and 50 mL of water. The organic layer was separated, dried with Na_2SO_4, filtered, and concentrated to 5 mL. The solution was applied to a column packed with silica gel (50 mL). Elution was performed with a linear gradient of acetone in dichloromethane (0–20%) containing 0.1% of pyridine. Appropriate fractions were pooled and evaporated to give 2′-azidomethylderivative **2d** as white foam. The ^{1}H NMR spectrum revealed that the substance was contaminated with triphenylphosphonium derivatives. An analytical sample was subjected repeated chromatography for characterization. R_f 0.53 (CH_2Cl_2:EtOH 9.5:0.5); ν_{max}/cm^{-1} 2099; δ_H (300 MHz, $CDCl_3$) 1.32 (3H, d, *J* 6.9, –CH_3(*i*Bu)), 1.33 (3H, d, *J* 6.9, –CH_3(*i*Bu)), 1.59 (1H, dd, *J* 10.0, 11.4, H5′a), 1.79 (1H, dd, *J* 9.9, 11.4, H3′a), 2.68 (1H), sep, *J* 6.9 (–CH < (*i*Bu)), 3.16 (1H, dt, *J* 11.5, 2.4, H5′e), 3.26 (1H, dd, *J* 13.3, 4.8, N_3CH_2–), 3.33 (1H, dd *J* 13.3, 6.0, N_3CH_2–), 3.49 (1H, dt, *J* 11.3, 2.4, H3′e), 4.32 (1H, m, H2′), 6.02 (1H, dd, *J* 10.0, 2.4, H6′), 7.18-7.60 (15H, m, Tr), 8.18 (1H, s, H8(Gua)); *m*/*z* 362.43 $[M-Tr+2H]^+$ (The Tr protective group was removed during the sample preparation). The crude product was dissolved in a mixture of pyridine (5 mL) and concentrated aqueous ammonia (2 mL). The reaction mixture was stirred 4 days at room temperature. After the base deprotection was complete, the solution was evaporated several times with water to remove traces of pyridine and the residue was dissolved in 80% aqueous acetic acid (10 mL). After 30 min, the reaction

mixture was evaporated several times with water; the residue was suspended in water containing TFA (0.1%) and filtered. The filtrate was applied to a column (2.5 × 15 cm) packed with Porasil C_{18} 55–105 μm (Waters, Milford, MA, USA). Chromatography was performed with a linear gradient of 300 mL each of 0.1% aqueous TFA and 10% ethanol in 0.1% aqueous TFA. Appropriate fractions were pooled and evaporated to give 2′-azidomethylderivative **4d** as a white powder (0.25 g, 85%). R_f 0.33 (*i*PrOH:H_2O 4:1); δ_H (400 MHz, D_2O) 3.40 (1H, dd, *J* 11.3, 13.0, H5′), 3.66 (2H, m, H3′, H5′), 3.80 (1H, dd, *J* 13.2, 3.6, H3′), 3.87–3.97 (2H, m, N_3CH_2–), 4.56 (1H, m, H2′), 6.23 (1H, dd, *J* 10.2, 4.0, H6′) 8.34 (1H, s, H8(Gua)); δ_C (100 MHz, D_2O) 46.17, 46.88, 54.27, 76.12, 80.41, 119.25, 139.80, 153.69, 157.84, 160.74. Compound **4d** (0.25 g, 0.85 mmol) was suspended in pyridine (3 mL), and triphenylphosphine (0.39 g, 1.5 mmol) was added to the reaction mixture. After 2 h of stirring at room temperature, concentrated aqueous ammonia (2 mL) was added, and the solution was left overnight with stirring. The reaction mixture was evaporated; the residue was suspended in water containing TFA (0.1%) and filtered. The filtrate was applied to a column (2.0 × 13 cm) packed with Servacel P-23 (Serva, Heidelberg, Germany) in the NH_4^+ form. Elution was performed with a linear gradient of 300 mL each of 20% ethanol in water and 0.075 M NH_4HCO_3 in 20% ethanol in water. Appropriate fractions were pooled and evaporated. The residue was coevaporated several times with ethanol to remove traces of ammonium bicarbonate and dried under vacuum. Compound **8** was obtained as a white powder (0.15 g, 0.46 mmol, yield 54%). Found: C, 40.85, H, 5.55, N, 30.50; calculated for $C_{10}H_{15}N_7O_2+H_2CO_3$: C, 40.4, H, 5.25, N, 29.95; R_f 0.32 (*i*PrOH:H_2O:conc.ammonia 6:3:1); δ_H (300 MHz, D_2O+2mcL TFA) 2.53 (1H, dd, *J* 10.0, 13.0, H5′), 2.90 (1H, dd, *J* 13.0, 2.5, H3′), 2.95–3.21 (4H, m, H3′,H5′, NH_2CH_2–), 4.06 (1H, m, H2′), 5.49 (1H, dd, *J* 10.0, 3.2, H6′), 7.77 (1H, s, H8(Gua)); δ_C (75.5 MHz, D_2O+2mkl TFA) 40.71, 44.87, 46.72, 73.61, 79.2, 115.58, 145.19, 150.65, 153.86, 158.61; *m*/*z* 266.64 $[M+H]^+$.

2.2.2. 2′-Aminomethyl-6′-(cytosine-1-yl)- morpholine 7 (Scheme 1)

Compound **7** was synthesized from **1c** following exactly the same reaction sequence as in the case of compound **8**. The target product was obtained as a white powder (0.10 g, 0.39 mmol, yield 39% starting from compound **1c**). Found: C, 44.2, H, 6.65, N, 27.6; calculated for $C_9H_{17}N_5O_2$ +1/2H_2CO_3: C, 44.2, H, 7.0, N, 27.1; R_f 0.37 (*i*PrOH:H_2O:conc.ammonia 6:3:1); δ_H (300 MHz, D_2O) 2.41 (1H, app. t, *J* 12.1, H5′), 2.56 (1H, app. t, *J* 11.5, H3′), 2.64–3.14 (4H, m, H3′, H5′, NH_2CH_2–), 3.79 (1H, m, H2′), 5.60 (1H, dd, *J* 10.0, 2.6, H6′), 5.90 (1H, d, *J* 7.5, H5(Cyt)), 7.63 (1H, d, *J* 7.5, H6(Cyt)); δ_C (100 MHz, D_2O) 45.85, 48.56, 50.39, 79.8, 84.45, 99.50, 145.04, 157.02, 169.33; *m*/*z* 228.51 $[M+H]^+$.

2.2.3. 2′-Aminomethyl-6′-(adenine-9-yl)-morpholine 6 (Scheme 1)

Compound **2b** was obtained from **1b** as described above for **2d**. R_f 0.69 (CH_2Cl_2:EtOH 9.5:0.5); ν_{max}/cm^{-1} 2101; δ_H (400 MHz, $(CD_3)_2CO$) 1.70 (1H, dd, *J* 10.5, 11.0, H5′a), 2.21 (1H, dd, *J* 9.9, 11.4, H3′a), 3.16 (1H, dt, *J* 11.0, 2.4, H5′e), 3.39 (1H, dd, *J* 13.0, 6.0, N_3CH_2–), 3.45 (1H, dd *J* 13.0, 4.0, N_3CH_2–), 3.60 (1H, dt, *J* 11.4, 2.4, H3′e), 4.68 (1H, m, H2′), 6.55 (1H, dd, *J* 10.5, 2.4, H6′), 7.16–7.72 (18H, m, Tr, *m*Bz, *p*Bz), 8.08 (2H, bd, *J* 7.6, *o*Bz), 8.25 (1H, s, H8(Ade)), 8.65 (1H, s, H2(Ade)); *m*/*z* 380.43 $[M-Tr+2H]^+$ (The Tr protective group was removed during the sample preparation). The crude product **2b** (1.6 g, 2.2 mmol) was dissolved in ethanol (25 mL), and 200 mg Pd/C (5%), was added.

The reaction mixture was stirred overnight at room temperature in a hydrogen atmosphere at positive hydrogen pressure (0.1 Bar). After the reduction was complete the reaction mixture was filtered, the precipitate was thoroughly washed with ethanol, the filtrate was evaporated, and the residue was dissolved in CH_2Cl_2 containing 0.1% of pyridine (20 mL). The solution was applied to a column packed with silica gel (50 mL). Elution was performed with a linear gradient of acetone in dichloromethane (0–70%) containing 0.1% of pyridine. Appropriate fractions were pooled and evaporated to give the 2′-aminomethyl derivative **3b** as white foam (1 g, 1.7 mmol, yield 64%). R_f 0.09 (CH_2Cl_2:EtOH 9:1); δ_H (400 MHz, $(CD_3)_2CO$) 1.58 (1H, bt, *J* 11.0, H5′a), 2.15 (1H, bt, *J* 10.0, H3′a), 3.15 (1H, dd, *J* 13.5, 6.5, NH_2CH_2–), 3.33 (1H, dd *J* 13.0, 5.0, NH_2CH_2–), 3.42 (1H, dt, *J* 11.0, 2.4, H5′e), 3.55 (1H, dt, *J* 10.0, 2.6, H3′e), 4.56 (1H, m, H2′), 6.47 (1H, dd, *J* 11.0, 2.4, H6′), 7.16–7.62 (18H, m, Tr, *m*Bz, *p*Bz), 8.07 (2H, bd, *J* 7.6, *o*Bz), 8.21 (1H, s, H8(Ade)), 8.62 (1H, s, H2(Ade)); *m*/*z* 354.38 $[M\text{-}Tr+2H]^+$ (The Tr protective group was removed during the sample preparation). Compound **3b** was used in the synthesis of **9** (X = N) and **12**. The sample of **3b** (0.3 g, 0.5 mmol) was dissolved in a mixture of pyridine (5 mL) and concentrated aqueous ammonia (2 mL). The reaction mixture was stirred for 2 days at room temperature. After the base deprotection was complete, the solution was evaporated several times with water to remove traces of pyridine and the residue was dissolved in 10 mL of 80% aqueous acetic acid. After 30 min, the reaction mixture was evaporated several times with water; the residue was suspended in water containing TFA (0.1%) and filtered. Cation exchange chromatography was performed as above. Compound **6** was obtained as a white powder (0.13 g, 0.42 mmol, and yield 84%). Found: C, 42.55, H, 5.5, N, 31.3; calculated for $C_{10}H_{15}N_7O+H_2\ CO_3$: C, 42.45, H, 5.5, N, 31.5; R_f 0.52 (*i*PrOH:H_2O:conc.ammonia 6:3:1); δ_H (400 MHz, D_2O) 2.72 (1H, app. t, *J* 12.3, H5′), 2.79–2.96 (2H, m, NH_2CH_2–), 3.00–3.14 (1H, m, H3′), 3.22–3.34 (2H, m, H3′,H5′), 4.04 (1H, m, H2′), 5.86 (1H, dd, *J* 8.7, 4.6, H6′), 8.21 (1H, s, H8(Ade)), 8.32 (1H, s, H2(Ade)); δ_C (100 MHz, D_2O) 42.30, 45.35, 46.93, 78.32, 80.03, 118.01, 139.69, 151.46, 152.47, 155.15; *m*/*z* 250.11 $[M+H]^+$.

2.2.4. 2′-Aminomethyl-6′-(uracil-1-yl)-morpholine 5 (Scheme 1)

Compound **5** was synthesized from **1a** following exactly the same reaction sequence as in the case of compound **6**, except for the ammonia treatment during the deprotection of intermediate **3a**. The target product was obtained as a white powder (0.16 g, 0.56 mmol, yield 56% starting from compound **1a**). Found: C, 41.2, H, 5.6, N, 20.25; calculated for $C_9H_{14}N_4O_3$ $+H_2CO_3$: C, 41,7, H, 5.6, N, 19.45; R_f 0.55 (*i*PrOH:H_2O:conc.ammonia 6:3:1); δ_H (300 MHz, D_2O) 2.44 (1H, dd, *J* 10.5, 13.1, H5′), 2.64 (1H, dd, *J* 10.5, 12.8, H3′), 2.75–3.00 (4H, m, H3′,H5′, NH_2CH_2–), 3.90 (1H, m, H2′), 5.62 (1H, dd, *J* 10.5, 2.6, H6′), 5.74 (1H, d, *J* 8.0, H5(Ura)), 7.65 (1H, d, *J* 7.5, H6(Ura)); δ_C (75.5 MHz, D_2O) 41.74, 45.17, 46.73, 76.57, 80.65, 102.42, 141.27, 154.96, 171.26; *m*/*z* 227.12 $[M+H]^+$.

2.2.5. p-Nitrophenyl 2′-hydroxymethyl-4′-trityl-6′-(N^6-Bz-adenine-9-yl)-morpholine sulfate 9 (Scheme 2, X = O)

Compound **9** (X = O) was synthesized from compound **1b** following the published procedure for aminonucleosides[8] in the presence of a considerable excess of *p*-nitrophenol. Yield 50%. δ_H (400 MHz, DMSO-[D]$_6$) 1.48 (1H, app. t, *J* 11.1, H5′), 2.10 (1H, app. t, *J* 10.5, H3′), 3.05–3.55 (4H, m, H3′,5′), 3.60 (1H, dd, *J* 10.0, 6.0, –SO_2OCH_2–), 3.73 (1H, dd, *J* 10.0, 5.0, –SO_2OCH_2–), 4.49 (1H, m, H2′), 6.41 (1H, dd, *J* 10.0, 2.8), 7.15–7.70

(20H, m, Tr, *m*Bz+*p*Bz), 2H(NO_2Ph–), 8.03 (2H, bd, *J* 8.0, *o*Bz), 8.19 (2H, d, *J* 9.0), 2H(NO_2Ph–), 8.45 (1H, s, H8(Ade)), 8.72 (1H, s, H2(Ade)); *m*/*z* 798,29 $[M+H]^+$.

2.2.6. 2′-Methoxyoxalylamidomethyl-4′-trityl- 6′-(N⁶-Bz- adenine-9-yl)-morpholine ***12*** *(Scheme 3)*

The 2′-Aminomethyl derivative **3b** (0.238 g, 0.5 mmol) was added to a stirred solution of dimethyl oxalate (0.236 g, 2 mmol) and TEA (0.28 mL, 2 mmol) in dichloromethane (2 mL). After 2 h, the reaction mixture was applied to a column packed with silica gel (50 mL). Elution was performed with a linear gradient of acetone in dichloromethane (0–20%) containing 0.1% of pyridine. Appropriate fractions were pooled and evaporated to give target compound **12** (0.12 g, 0.18 mmol, 36 %) as a white foam after drying under vacuum. R_f 0.67 (CH_2Cl_2:EtOH 9.5:0.5); δ_H (400 MHz, $(CD_3)_2CO$) 1.62 (1H, dd, *J* 10.0, 11.7, H5′a), 2.08 (1H, m, H3′a), 3.32 (1H, dt, *J* 11.0, 2.4, H5′), 3.37 (1H, dd *J* 12.8, 6.2, –NHCH_2–), 3.44–3.51 (1H, m, –NHCH_2–), 3.58 (1H, dt, *J* 11.5, 2.6, H3′e), 3.76 (3H, s, CH_3O–), 4.56 (1H, m, H2′), 6.48 (1H, dd, *J* 10.0, 2.4, H6′), 7.16–7.72 (18H, m, Tr, *m*Bz, *p*Bz), 8.09 (2H, bd, *J* 7.4, *o*Bz), 8.16 (1H, bt, *J* 5.7, –N*H*–), 8.27 (1H, s, H8(Ade)), 8.64 (1H, s, H2(Ade)); *m*/*z* 440,49 $[M\text{-}Tr+2H]^+$ (The Tr protective group was removed during the sample preparation).

2.2.7. 2′-O-(4,4′-Dimethoxytrityl)-hydroxymethyl- 4′- methoxyoxalyl-6′-(uracil-1-yl)-morpholine ***13*** *(Scheme 3)*

The activated monomer **13** was prepared in a manner similar to the activated monomer **12**, except that a prolonged reaction time (16 h) was applied. Yield 65%. R_f 0.60 (CH_2Cl_2:EtOH 9.5:0.5); δ_H (400 MHz, $(CD_3)_2CO$) 2.96 and 3.07 (0.5H each, 2dd, *J* 10.9, 13.2, 10.3, 13.1 H5′), 3.15–3.38 (2.5 H, m, H3′, –OCH_2–), 3.45 (0.5H, dd, *J* 10.2, 13.2, H3′), 3.78 (6H, s, CH_3O-(DMTr)), 3.84 and 3.87 (1.5H each, 2s, CH_3O-(Ox)), 3.89 and 3.92 (0.5H each, dm, *J* 13.5, H5′) 4.09 (1H, m, H2′), 4.44 and 4.54 (0.5H each, 2dm, *J* 13.3, 12.7, H3′), 5.67 and 5,69 (0.5H each, 2d, *J* 8.0, H5(Ura)), 5.74 and 5.77 (0.5H each, 2dd, *J* 8.5, 2.7, H6′), 6.88 (4H, bd, *J* 8.6, DMTr), 7.20–7.52 (9H, m, DMTr), 7.71 and 7.73 (0.5H each, 2d, *J* 8.0, H6(Ura)); *m*/*z* 314.12 $[M\text{-}DMTr+2H]^+$ (The DMTr protective group was removed during the sample preparation).

2.2.8. 2′-Hydroxymethyl-4′-methoxyoxalyl-6′-(uracil-1-yl)-morpholine ***15*** *(Scheme 4)*

Activated monomer **15** was prepared from monomer **14** similar to that of activated monomer **13**. Dimethylformamide was used as a solvent. A linear gradient of acetone in dichloromethane (0–60%) was applied over the course of silica gel chromatography. Yield 65%. R_f 0.20 (CH_2Cl_2:EtOH 9.5:0.5). δ_H (300 MHz, D_2O) 2.87 and 2.99 (0.5H each, 2dd, *J* 13.5, 12.0, 13.0, 10.5, H5′), 3.21 and 3.33 (0.5 H each, 2dd, 13.3, 11.7, 13.5, 10.0, H3′), 3.54–3.70 (2H, m, –OCH_2–), 3.76 (0.5H, dm, *J* 12.5, H5′), 3.80 and 3.82 (1.5H each, 2s, CH_3O–), 3.90 (1H, m, H2′), 3.96 (0.5H, dm, *J* 13.5, H5′), 4.20 and 4.37 (0.5H each, 2m, H3′), 5.68 and 5.71 (0.5H each, 2dd, *J*10.7, 2.6, H6′), 5.77 and 5.79 (0.5H each, 2d, *J*8.2, H5(Ura)), 7.73 and 7.75 (0.5H each, 2d, *J* 8.2, H6(Ura)); *m*/*z* 314.12 $[M+H]^+$.

2.2.9. 2′-Hydroxymethyl-6′-(uracil-1-yl)-morpholine-4′-oxalyl-2′-N-[2′-aminomethyl-6′-(uracil-1-yl)-morpholine] ***16*** *(Scheme 4)*

2′-Hydroxymethyl-4′-methoxyoxalyl-6′-(uracil-1-yl)-morpholine **15** (0.031 g, 0.1 mmol) and 2′-aminomethylderivative **5** (0.058 g, 0.2 mmol) were dissolved in dry pyridine

(0.5 mL); TEA (0.1 mL, 0.7 mmol) was added to the reaction mixture and the solution was stirred overnight. The reaction progress was monitored by TLC and analytical HPLC. After the coupling was complete, the solution was coevaporated several times with water to remove pyridine, and the residue was dissolved in 0.1% aqueous TFA. The target product was purified by reverse phase HPLC in a gradient of acetonitrile (0–10%) in 0.1% aqueous TFA. Appropriate fractions were pulled and evaporated. The residue was dried under vacuum and re-dissolved in water. The concentration of dimer stock solution was determined spectrophotometrically. Aliquots of this stock solution were used in recording of NMR, CD-spectra, melting experiments, and in other experiments. Yield 160 AU_{260} (0.08 mmol, 80%). R_f 0.38 (*i*PrOH:H_2O 4:1). δ_H (400 MHz, D_2O) 2.93–3.05 (1H, m, H(Mor)), 3.08–3.18 (1H, m, H(Mor)), 3.27–3.45 (1H, m, H(Mor)), 3.50–3.89 (7H, m, H(Mor)), 3.90–4.05 (2H, m, H(Mor)), 4.29–4.53 (2H, m, H(Mor)), 5.77–5.88 (1H, m, H6′), 5.92, 5.93, 5.95 (0.5H, 0.5H and 1H, 3d, *J* 8.0, H5(Ura)), 6.03–6.13 (1H, m, H6′), 7.77 and 7.78 (O.5H each, 2d, *J* 8.0, H6(Ura)), 7.88 and 7.89 (O.5H each, 2d, *J* 8.0, H6(Ura)); *m*/*z* 508.62 $[M+H]^+$.

2.2.10. 2′-Hydroxymethyl-6′-(uracil-1-yl)- morpholine-4′-oxalyl-2′-N-[2′-aminomethyl-6′-(adenine-9-yl)-morpholine] **17** *(Scheme 4)*

Dimer HOMorU-Ox-NHMorA **17** was synthesized and purified exactly as described for dimer **16**. Monomer **6** was used as the amino component. Yield 220 AU_{260} (0.088 mmol, 88%). R_f 0.24 (*i*PrOH:H_2O 4:1). δ_H (400 MHz, D_2O) 3.08 and 3.20 (0.6H and 0.4H, 2dd, *J* 13.5, 11.0, H(Mor)), 3.31–3.51 (2H, m, H(Mor)), 3.69–4.12 (9H, m, H(Mor)), 4.39–4.66 (2H, m, H(Mor)), 5.82–5.89 (1H, m, H6′), 5.98 and 6.04 (0.6H and 0.4H, 2d, *J* 8.0, H5(Ura)), 6.42 and 6.48 (0.6H and 0.4H, 2dd *J* 11.0, 2.4 H6′), 7.93 and 7.97 (0.6H and 0.4H, 2d, *J* 8.0, H6(Ura)), 8.58, 8.59, 8.61, 8.67 (0.6H, 0.6H, 0.4H, 0.4H, 4s, H2 and H8(Ade)); *m*/*z* 531.42 $[M+H]^+$.

2.2.11. Hexamer HOMorU-(Ox-NHMorU)$_5$ **18** *(Scheme 5)*

TEA (0.28 mL, 2 mmol) was added to a solution of activated monomer **13** (0.135 g, 0.22 mmol) and amino component **5** (0.19 g, 0.66 mmol, bicarbonate salt) in pyridine (2 mL). The reaction mixture was stirred at room temperature overnight, then distributed between 20 mL of dichloromethane and 20 mL of water. The organic layer was dried over Na_2SO_4 and evaporated; the residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a tenfold volume of light petroleum ether. Yield 0.16 g (0.20 mmol, 91%). The aqueous layer was evaporated, and the residue was dried by coevaporation with dry acetonitrile and used in the following coupling steps as amino component. The structure of dimer DMTr-*O*-MorU-Ox-NHMorU obtained after the first condensation step was confirmed by NMR and mass spectral data (see Table 1). Dimer DMTr-*O*-MorU-Ox-NHMorU (0.15 g, 0.19 mmol) and dimethyl oxalate (0.35 g, 3 mmol) were dissolved in pyridine (2 mL), and TEA (0.42 mL, 3 mmol) was added to the solution. The reaction mixture was stirred for 24 h. After the reaction was complete, the reaction mixture was evaporated, the residue was diluted with dichlorometane, and the solution was applied to a column packed with silica gel (30 mL). Elution was performed with a linear gradient of methanol in dichloromethane (0–15%) containing 0.1% of pyridine. Appropriate fractions were pooled and evaporated to produce the activated dimer, DMTr-*O*-MorU-Ox-NHMorU-Ox-OCH_3 (0.14 g, 0.16 mmol, 84%). The total yield of the condensation and activation steps was 76%. The coupling and activation steps were

Table 1
Mass spectral data and the integral ratio of some proton signals for uracil containing morpholine based oligomers

Compound	[M+H], DMTr-Deprotected fragment[a]	The integral ratio of H6(Ura)/four aromatic DMTr-protons at 7.00–6.90 ppm/H6′(Mor)+H5(Ura)/OCH_3(DMTr)/OCH_3(methoxyoxalate, if present)[b]
DMTr-*O*-MorU-Ox-NHMorU	508.14	0.50/1.00/1.10/1.57/—
DMTr-*O*-MorU-Ox-NHMorU-Ox-OCH_3	594.20	0.53/1.00/0.93/1.56/0.71
DMTr-*O*-MorU-(Ox-NHMorU)$_2$	788.52	0.78/1.00/1.69/1.48/—
DMTr-*O*-MorU-(Ox-NHMorU)$_2$-Ox-OCH_3	874.53	0.78/1.00/1.51/1.49/0.87
DMTr-*O*-MorU-(Ox-NHMorU)$_3$	1068.65	1.04/1.00/2.02/1.68/—
DMTr-*O*-MorU-(Ox-NHMorU)$_3$-Ox-OCH_3	1154.80	0.91/1.00/2.25/1.65/0.61
DMTr-*O*-MorU-(Ox-NHMorU)$_4$	1349.26	1.30/1.00/2.84/1.59/—
DMTr-*O*-MorU-(Ox-NHMorU)$_4$-Ox-OCH_3	1434.00	—
DMTr-*O*-MorU-(Ox-NHMorU)$_5$	1629.26	—
HOMorU-(Ox-NHMorU)$_5$ (**18**)	1629.56	—

[a] DMTr-protective group was removed during the sample preparation.
[b] Spectra were recorded in [D_4]-methanol, with several drops of [D_6]-DMSO.

repeated 4 and 3 times, respectively, with following modifications: the duration of the coupling steps was increased to 24–48 h; after the coupling step and before the extraction, the evaporated reaction mixture was suspended in the CH_2Cl_2:H_2O:CH_3CH_2OH (4:5:1) mixture; and the organic layer after the extraction was concentrated and subjected to silica gel chromatography performed with a linear gradient of methanol (0–60%) in dichloromethane containing 0.1% of pyridine. The structure of all intermediate oligomers and activated derivatives was confirmed by NMR and mass spectral data (see Table 1). Fifteen milligrams of DMTr-*O*-MorU-(Ox-NHMorU)$_5$ was obtained (0.008 mmol, total yield 3.6% starting from monomer **13**, 70% per each step). DMTr-*O*-MorU-(Ox-NHMorU)$_5$ (10 mg, 0.005 mmol) was dissolved in 80% aqueous acetic acid (1 mL). After 40 min, the reaction mixture was diluted ten fold with water and evaporated. The residue was dissolved in 0.1% aqueous TFA. The target product was purified by reverse phase HPLC in a gradient of acetonitrile (0–30%) in 0.1% aqueous TFA. Appropriate fractions were pulled and evaporated. The residue was dried under vacuum and re-dissolved in water. The concentration of hexamer stock solution was determined spectrophotometrically. Yield 150 AU_{260} (2.5 μm, 50%). ^{1}H NMR and mass spectral data for the intermediates and the final product are summarized in Table 1.

2.3. Stability of morpholine oxalyl diamide dimers in different solutions

To study the stability of dimers **16** and **17**, the aliquots of stock aqueous solutions containing 1–2 AU_{260} of analogues were lyophilized, then dimers were dissolved in 0.1 mL of corresponding solvent (25% aqueous ammonia, 80% aqueous acetic acid, 6 N methanolic ammonia or aqueous buffers). Aliquots of reaction mixtures (0.01 mL) were withdrawn at the appropriate time and analyzed by analytical reverse phase HPLC. The reaction progress was estimated by the disappearance of starting compound. The reaction products were identified by comparison with authentic samples.

2.4. UV studies

The concentration of aqueous solutions of oligonucleotides and analogues at 95 °C were determined spectrophotometrically at 260 nm. The extinction coefficients of di- and oligonucleotide analogues were calculated using the sum of the extinction coefficients of corresponding nucleosides (15.02 $mM^{-1}cm^{-1}$ for A, 9.66 for U) [20]. To determine the concentrations of poly(dA) and poly(rA), ε_{260} values per nucleotide residue 8.4 and 10.3 mM^{-1} cm^{-1} were used, respectively [21].

Electronic spectra of samples were registered using 0.2 cm path length quartz cell on a Cary 300 Bio UV–Visible spectrophotometer (Varian, Palo Alto, Ca, USA) equipped with a peltier thermostable multicell (6 × 6) holder in water. All spectra were recorded from 200 to 330 nm with 1 nm resolution. The samples of dimers **16** and **17** were treated with 6 N methanolic ammonia to give the mixture of the monomer HOMorU and the corresponding oxalylamide derivatives, NH_2-Ox-NHMorB (B = Ura or Ade). After evaporation of the reaction mixture, the residues were re-dissolved in water and the electronic spectra were recorded for the mixtures of corresponding monomers in the same temperature region.

CD spectra were registered at 12–85 °C using 0.1 cm path length quartz cell on a JASCO J-600 spectropolarimeter (JASCO Co., Ltd., Japan) connected to a LKB 2219

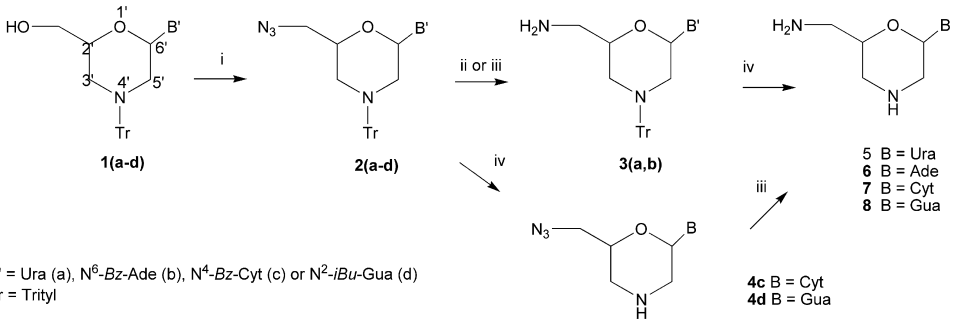
MULTITHERM II water bath (LKB, Bromma, Sweden). All CD spectra were performed in triplicate from 200 to 330 nm with 0.2 nm resolution, and the average values are presented. The samples were dissolved in 10 mM Na-phosphate buffer adjusted to pH 7.25 containing 0.2 M NaCl and 0.1 mM EDTA. Dimer concentration was 0.83 mM (compound **16** and a mixture of corresponding monomers) and 0.59 mM (compound **17** and a mixture of corresponding monomers). The samples of dimers **16** and **17** were treated with 6 N methanolic ammonia to give the mixture of monomer HOMorU and corresponding oxalylamide derivatives NH_2-Ox-NHMorB (B = Ura or Ade). After evaporation of the reaction mixture, the residues were dissolved in the buffer used for CD experiments, and CD spectra were recorded for the equimolar mixture of corresponding monomers.

Melting of the oligomucleotide complexes was carried out in a thermo regulated cell equipped with a special device based on the UV detector of the Milichrom liquid chromatograph (Econova, Novosibirsk, Russia) as described earlier [22]. Each melting curve comprised at least 600 absorbance values with a frequency of 10 points/°C and was recorded with a heating rate of 0.7–1 °C/min. The heating curves coincided with cooling curves, indicating that the formation of the complexes was under thermodynamic equilibrium. The recording of the melting curves in the multi wavelength mode was carried out by automatic switching of the monochromator between four wavelengths in the range 230–300 nm. The integration time for the absorbance value at each wavelength did not exceed 1.2 s. The complexes were formed by stoichiometric mixing of the oligonucleotide and/or polynucleotide strands, with the total concentration of the complementary nucleotide residues 1.2 mM in 10 mM Na-phosphate buffer adjusted to pH 7.25 containing 0.2 M NaCl and 0.1 mM EDTA.

3. Results and discussion

3.1. The choice of tether group

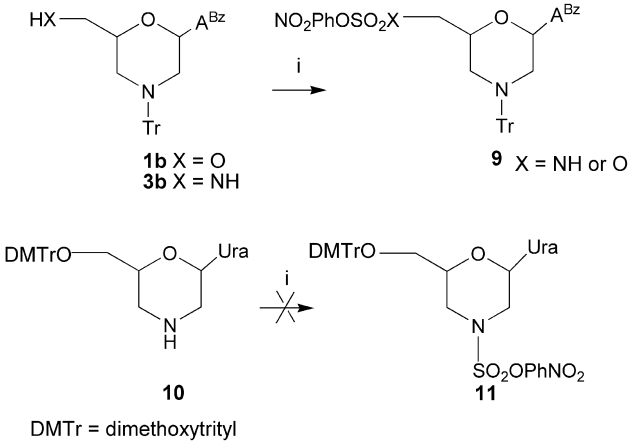
Morpholine nucleosides, introduced by J. Summerton and D. Weller [18,23], initially attracted our attention as a basis for the synthesis of modified triphosphates and some triphosphate derivatives [24,25]. We were also interested in oligonucleotide analogues synthesis. We sought to obtain the achiral morpholine oligonucleotide analogues proposed earlier [18] using *p*-nitrophenyl chlorosulfate as an activating agent, which was recently successfully used in the synthesis of sulfamate and sulfamide linked oligonucleotide analogues [8]. The use of *p*-nitrophenyl chlorosulfate promised to considerably simplify the synthesis of the morpholine oligomer in comparison with the published procedure [18]. 2′-Aminomethyl derivatives from suitably protected morpholine nucleosides necessary for these experiments were synthesized as shown in Scheme 1, starting from *N*-(morpholine)-and base-protected monomers **1(a–d)** obtained according to the literature [18]. The treatment of the protected morpholine nucleosides **1(a–d)** with triphenylphosphine, sodium azide, and bromotrichloromethane in DMF, similar to the published approach [26], produced 2′-azidomethyl morpholine nucleosides **2(a–d)**. The subsequent reduction of the azido group by H_2 over a Pd/C catalyst or in the presence of triphenylphosphine [27] gave protected aminoderivatives **3(a, b)** from **2(a, b)**. Since the purification of protected azidoderivatives **2(a–d)** using silica gel chromatography was not very effective, the reduction and deprotection steps were exchanged, in the case of the cytosine and guanosine derivatives as depicted in Scheme 1, to facilitate the purification of the 2′-azidomethyl



Scheme 1. Synthesis of 2′-aminomethyl derivatives of morpholine nucleosides: (i) $Ph_3P/CBrCl_3$, NaN_3, DMF; (ii) H_2/Pd/C, MeOH; (iii) Ph_3P, Py, then aqueous ammonia; (iv) Py/aqueous ammonia, then $HOAc/H_2O$.

morpholine nucleosides. After deprotection, the 2′-aminomethylmorpholine nucleosides **5–8** were purified using cation-exchange chromatography.

Then, we tried to obtain activated monomers **9** and **11** (Scheme 2) using *p*-nitrophenyl chlorosulfate under the published conditions [8]. We failed to obtain the compound **11**; we identified two main products formed in the reaction mixture as the triethylammonium salt of its corresponding sulfamic acid and a symmetric dimer on the basis of NMR and mass spectroscopy data. On the other hand, *p*-nitrophenyl-*O*-sulfo-4′-*N*-morpholide **11**, if obtained, would not have been able to form an indispensable active *N*-sulfonylamine intermediate discussed earlier [8]. As for the activated derivative **9**, if X = NH, sulfamate **9** was found to be too unstable, and decomposed in solution or in the reaction mixture, producing the corresponding amino derivative **3b**. We did not manage to isolate the homogenous substance in this case. If X = O, the sulfate diester was found to be inactive in the coupling reaction with morpholine nucleoside. When ethylene diamine or histamine was used in model coupling experiments instead of morpholine nucleoside as the amino component, we observed the partial cleavage of the sulfo-group with simultaneous deprotection of



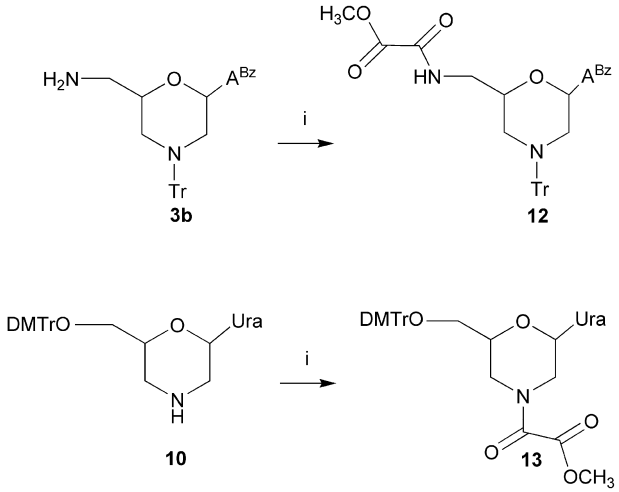
Scheme 2. Synthesis of *p*-nitrophenyl sulfate and sulfamate morpholine derivatives: (i) *p*-nitrophenyl chlorosulfate, *p*-nitrophenol, TEA, CH_2Cl_2.

adenine heterocycle. So, we did not succeed in obtaining any dimer with the sulfo-group as a tether using *p*-nitrophenyl as the leaving group.

However, the obvious difference in the reactivity of the primary 2′-aminomethyl group and the morpholine nitrogen of morpholine nucleoside motivated us to develop a synthetic strategy based on the different reactivities of these functions. The best choice would be to find the bifunctional reagent capable of differentiating between two reaction centers. In this case, the use of a temporary protective group (for example, *N*-(morpholine)-trityl) would not be necessary, and the synthesis could be considerably facilitated.

In search of other linkers joining monomer nucleoside subunits, we decided to use an oxalyl residue. The dimethyl ester of oxalic acid readily reacts with aliphatic amino groups and is widely used as a bifunctional reagent in precursor-based approaches in the course of obtaining oligonucleotide conjugates [28,29]. Also, it is important that the oxalic acid tether does not introduce additional chiral centers in the oligomer. As shown in numerous studies, the absolute configuration of the intersubunit chiral center of oligonucleotide analogues (phosphotriesters, methylphosphonates, phosphorothioates) dramatically influences the stability of their complementary complexes [30]. The last generation oligonucleotide analogues also exhibits this tendency [31].

We synthesized the activated derivatives **12** and **13**, treating corresponding monomers with a dimethyl oxalate excess (Scheme 3). Compound **3b** reacted faster than compound **10** due to the higher reactivity of the primary amino group than the morpholine nitrogen towards dimethyl oxalate. The steric hindrance of the morpholine cycle should also influence the progress of the coupling reaction on the dimer formation step. So we anticipated the coupling reaction proceeds between derivatives **13** and **3b** rather than **12** and **10**. In model experiments, we proved that this assumption was true. We did not notice considerable dimer formation mixing compounds **12** and **10,** while coupling between **13** and **3b** was found to be successful. We also discovered that the difference in reactivity of the primary 2′-aminomethyl group and the morpholine nitrogen was so significant that it was possible to accomplish the coupling reaction of derivative **13** and the amino component without the

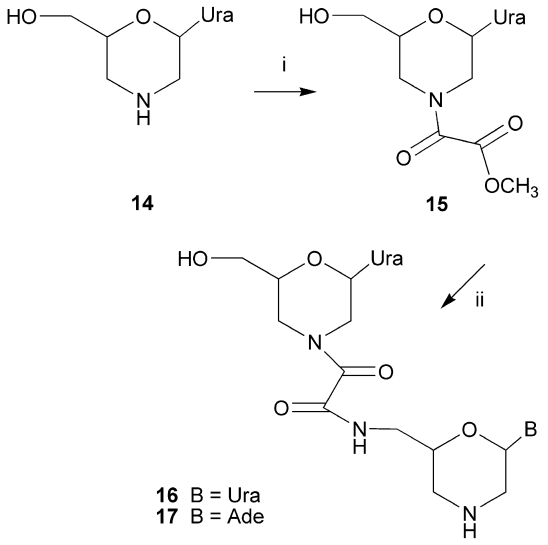


Scheme 3. Synthesis of methoxyoxalylamido morpholine derivatives: (i) dimethyl oxalate, TEA, CH_2Cl_2.

morpholine nitrogen protection. In this case, the dimer formation was the most effective. So, methoxyoxalyl-activated morpholine nucleoside derivatives selectively react with the primary 2′-aminomethyl group of the second component during the coupling reaction. This property of methoxyoxalyl-activated morpholine nucleoside derivatives would allow simplification of the activation step and coupling reaction in the course of oligomer synthesis, especially in a solid phase variant.

3.2. The synthesis of morpholidooxalylamido oligomers

For the purpose of a preliminary study of the properties of oxalyl diamide-linked morpholine oligonucleotide analogues, we synthesized dimers **16** and **17** (Scheme 4). The most suitable solvent for the coupling reaction was found to be pyridine. When the coupling reaction was performed in methanol, the desired products, **16** or **17**, underwent a slow methanolysis, yielding **14** and the corresponding methoxyoxalyl amidomethyl derivative of the second component. Dimers **16** and **17** were purified by reverse phase chromatography and were found to be stable in aqueous solution in acidic and neutral pH, but readily hydrolyzed ($\tau_{1/2}$ 15 min) in concentrated aqueous ammonia giving rise to the mixture of **14,** 2′-amidooxalylamide and the 2′-carboxyoxalylamido derivative of the second monomer. The reaction progress was monitored by analytical HPLC. In 6 N methanolic ammonia, compound **14** and the 2′-amidomethyloxalylamide of the corresponding morpholine nucleoside were formed ($\tau_{1/2}$ 5 h). The instability of the morpholidooxalylamido linkage to ammonolysis would preclude using morpholine nucleosides with acyl protected nucleobases in the synthesis of morpholidooxalylamido linked oligonucleotide analogues. Luckily, dimethyl oxalate does not react with heterocyclic amino groups of nucleobases in any considerable extent, even over prolonged treatment (>5 days), so the activation

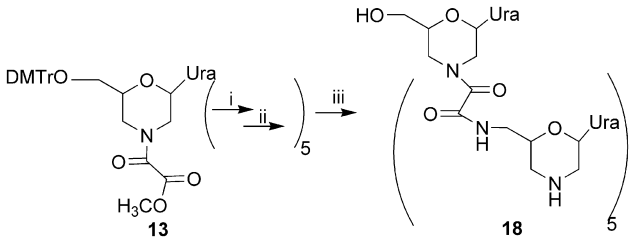


Scheme 4. Synthesis of morpholidooxalylamido morpholine dimers HOMorU-Ox-NHMorU (**16**) and HOMorU-Ox-NHMorA (**17**): (i) dimethyl oxalate, TEA, DMF; (ii) **5** or **6**, TEA, pyridine.

and elongation steps do not require nucleobase protection. We plan to use deprotected 2′-aminomethyl derivatives **5**–**8** for the oligonucleotide analogue synthesis in future.

Following the reaction sequence depicted in Scheme 5, we synthesized hexamer **18**. At least three fold excess of the amino component was used at each condensation step. The dimethoxytrityl (DMTr) protective group at the 2′-hydroxymethyl group of the starting monomer was introduced to facilitate the purification of intermediate oligomers. After the coupling step, a separation of the product from an excess of amino component was achieved by distributing the reaction mixture between water and dichloromethane. Evaporation of the aqueous layer and drying of the residue afforded a regenerated amino component, which could be used in the next cycle without purification. After treatment of the growing oligomer with an excess of dimethyl oxalate, the activated compound was purified by silica gel chromatography. After each elongation step, the structure of the elongated product was confirmed by mass spectroscopy (the dimethoxytrityl protective group was removed during the preparation of the analytic samples due to the use of dihydroxybenzoic acid as a matrix for MALDI-TOF mass spectra). The length of the oligomer can also be ascertained by comparing the integration of ^{1}H NMR signals, namely, the multiplet of H6(Ura) protons at 7.80–7.68 ppm, the well-separated dt-group of four aromatic DMTr protons at 7.00–6.90 ppm, the multiplets of H6′(Mor) and H5(Ura) at 5.85–5.65 ppm, and a singlet of six methoxy protons of the DMTr-residue at 3.75–3.85 ppm. The integrity of the products after the activation step was shown by mass spectroscopy, as well as the above mentioned proton integral ratios and additional methoxy protons at 3.85–3.95 ppm assigned to the methoxyoxalyl residue. It is necessary to mention that joining the methoxyoxalyl residue to the morpholine nitrogen resulted in the appearance of duplicate signals for the uracil, morpholine and methoxyoxalyl protons in the PMR spectra even at the monomer level (see Experimental for compounds **13** and **14**); therefore, methoxyoxalyl protons in the ^{1}H-NMR spectra of activated oligomers appeared as several (2–3) singlets. Mass spectral characteristics and integral ratios for the above mentioned proton groups from intermediate products are summarized in Table 1.

After acidic treatment of the DMTr-*O*-protected hexamer, the target hexamer **18** was purified by reverse phase chromatography in the presence of trifluoroacetic acid (TFA). Appropriate fractions were concentrated to dryness and dissolved in water. Dimers **16**, **17,** and hexamer **18** were found to be soluble up to millimolar concentrations in water and pH 7.5 buffer, so we had no difficulties in subsequent complementary duplex melting experiments and CD-spectra recording.



Scheme 5. Synthesis of morpholidooxalylamidomethylmorpholine hexamer HOMorU-(Ox-NHMorU)$_5$ **18**: (i) **5**, TEA, pyridine; (ii) dimethyl oxalate, TEA, pyridine; (iii) HOAc/H_2O.

3.3. UV studies of dimers **16** and **17** and complementary duplex formation of hexamer **18**

The electronic spectra of dimers **16** and **17** were recorded in the region from 200 to 320 nm in water. In both cases the shapes of the spectra are similar (dimer **16**: 258 nm (λ_{max}), 234 nm (λ_{min}); dimer **17**: 258 nm (λ_{max}), 230 nm (λ_{min})) and close to the spectra of the native nucleic acid dimers (data not shown). Only a slight long wavelength shift (1–2 nm) was observed when the temperature was increased from 25 to 95 °C without significant changes in the signal amplitude. Similar spectral characteristics were also observed for the mixture of monomer HOMorU and oxalylamide derivatives NH_2-Ox-NHMorB (B = Ura or Ade) obtained after the treatment of the corresponding dimers **16** and **17** with 6 N methanolic ammonia (see Section 2). In both cases, the electronic spectra of aqueous solutions of the monomer mixtures shifted to the long wavelength (1–2 nm) in comparison with the corresponding dimer spectra recorded at the same temperature. In all cases the spectral changes were fully reversible and reproducible depending on the temperature.

To investigate the ability of dimers **16** and **17** to adopt a base stacked conformation, circular dichroism spectroscopy was employed. CD spectra of some morpholine nucleoside dimers were studied earlier and the base stacking in uncharged phosphate-containing morpholine adenosine dimers was found [32]. In our study, we have revealed that the shapes of the CD spectra of dimers HOMorU-Ox-NHMorU **16** and HOMorU-Ox-NHMorA **17** have more similarities with the spectra of corresponding ribodinucleoside phosphates rather than deoxyribodinucleoside phosphates [33,34]. In both cases, the positive bands are close to 270 nm and the negative bands are observed at wavelengths <250 nm (Fig. 2). The intensity of the CD signals of the dimers are significantly higher in comparison with an equimolar mixture of the corresponding monomers of HOMorU and oxalylamide derivatives NH_2-Ox-NHMorB (B = Ura or Ade) obtained after the ammonolysis of compounds **16** and **17**, as mentioned above. The amplitudes of the CD spectra of dimers decrease as the temperature is increased. On the contrary, the CD spectra of the monomer mixtures do not change as temperature is increased from 25 to 85 °C. The data obtained indicate that the bases of the morpholine dimers **16** and **17** exist in a base-stacked conformation [33,34].

To investigate the thermal stability of the duplex of hexamer **18**, we chose oligonucleotides d($C_2A_6C_2$), poly(dA) and poly(rA) as a complementary strands. For comparison, complementary complex formation of dT_6 under the same conditions was studied. dT_6 forms complementary duplexes in all cases (T_m 10, 23 and 17 °C, respectively), but the complementary duplex formation between hexamer **18** and any of the complementary strand used was not detected; some hyperchromicity, however, was observed. Some typical melting curves obtained from thermal denaturation of the complementary duplexes are shown in Fig. 3. The length of the morpholine oligomer was probably not sufficient to form stable complexes under such conditions. Further studies are necessary to elucidate complex formation of morpholine oxalyl diamide oligonucleotide analogues having different lengths and base composition.

4. Conclusion

Morpholine nucleoside based oligomers are now among the more promising oligonucleotide analogues as potential theurapeutic agents [35–37]. We propose a new type of intersubunit linkage suitable to construct morpholine oligonucleotides with minimal effort

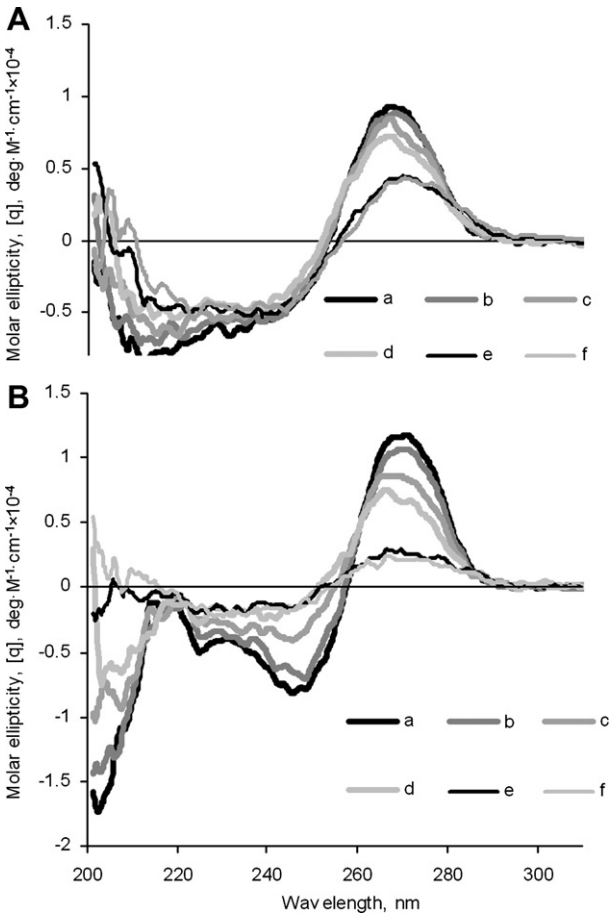


Fig. 2. Series of CD spectra (molar ellipticity per base *vs* wavelength) as a function of the temperature: (A) dimer **16** 12 °C (a), 25 °C (b), 55 °C (c), 85 °C (d) and mixture of corresponding monomers at 25 °C (e) and 85 °C (f); (B) dimer **17** 12 °C (a), 25 °C (b), 55 °C (c), 85 °C (d) and mixture of corresponding monomers at 25 °C (e) and 85 °C (f).

and cost. Our synthetic strategy uses the selective reaction of methoxyoxalyl-activated morpholine nucleoside derivatives with the primary 2′-aminomethyl group of the second component during the coupling reaction. This property allows us to not introduce the temporary trityl protective group on the morpholine nitrogen of the amino component. Furthermore, dimethyl oxalate does not react with aromatic amino groups of heterocyclic bases. So, the protection of heterocyclic bases is not necessary when using dimethyl oxalate during the activation step. To illustrate the abilities of our approach, we synthesized the dimers HOMorU-Ox-NHMorU, HOMorU-Ox-NHMorA and the uracil containing hexamer, HOMorU-(Ox-NHMorU)$_5$. Structures of the oligomers obtained were confirmed by NMR and mass spectroscopy. CD spectroscopy of the dimer solutions and the corresponding monomer mixture solutions suggest the presence of base stacking interactions in dimers contrary to the monomer mixture. In the future, we hope to develop an effective synthetic procedure to obtain the necessary 2′-aminomethyl monomers and to apply the chemistry used in this paper to the solid phase variant of oligomer synthesis.

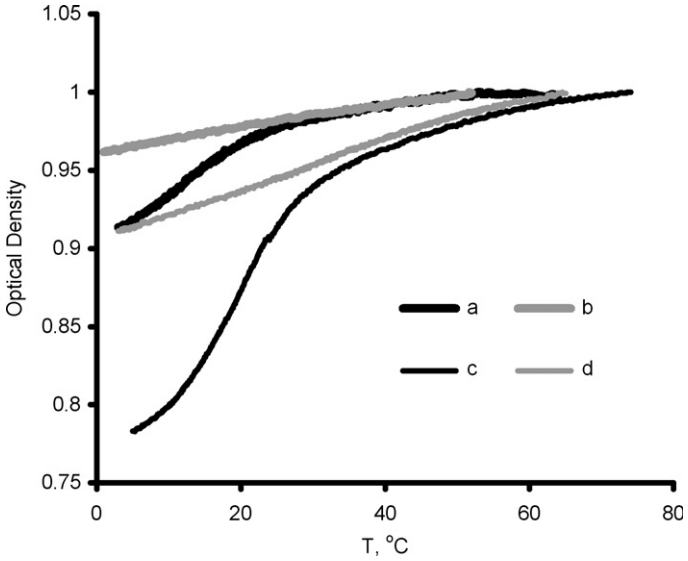


Fig. 3. Typical normalized integral melting curves; (a) dT_6 + $d(CA_6C)$ (270 nm); (b) hexamer **18** + $d(CA_6C)$ (270 nm); (c) dT_6 + poly(rA) (240 nm); (d) hexamer **18** + poly(rA) (240 nm).

Acknowledgments

We thank Dr. V. Koval for recording the MALDI mass spectra. This work was supported by the Fundamental Research Programs of RAS ''The origin of life and evolution of biosphere'', ''Fundamental research for medicine'' and ''Molecular and cell biology''.

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