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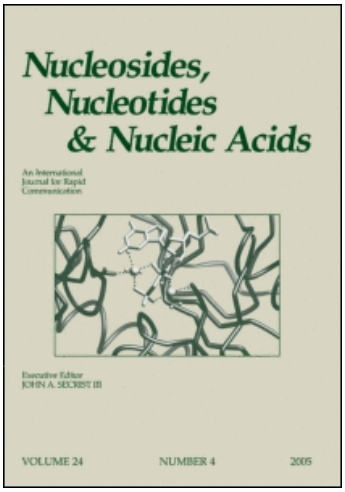

Nucleosides, Nucleotides and Nucleic Acids
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4-Aminometyl-3-Nitrobenzoic Acid—A Photocleavable Linker for Oligonucleotides Containing Combinatorial Libraries

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To cite this Article Abramova, Tatiana V. and Silnikov, Vladimir N.(2005) '4-Aminometyl-3-Nitrobenzoic Acid—A Photocleavable Linker for Oligonucleotides Containing Combinatorial Libraries', Nucleosides, Nucleotides and Nucleic Acids, 24: 9, 1333 — 1343
To link to this Article: DOI: 10.1080/15257770500230509
URL: http://dx.doi.org/10.1080/15257770500230509

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Nucleosides, Nucleotides, and Nucleic Acids, 24 (9):1333–1343, (2005)
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ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1080/15257770500230509

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4-AMINOMETYL-3-NITROBENZOIC ACID—A PHOTOCLEAVABLE LINKER FOR OLIGONUCLEOTIDES CONTAINING COMBINATORIAL LIBRARIES

Tatiana V. Abramova and Vladimir N. Silnikov ▫ *The Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia*

▫ *We detail the design, synthesis, and characterization of an o-nitrobenzyl–based photolabile linker containing amine and carboxyl anchor groups. A model nucleoside monomer modified with an imidazole residue and a precursor unit linked to a heterocyclic base through a photolabile tether is constructed. Upon UV irradiation (313–365 nm), the imidazole containing part of this molecule is released.*

Keywords Modified Nucleosides, Photolabile Linker, Combinatorial Libraries

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INTRODUCTION

The design and synthesis of a small synthetic molecule that mimics the active center of a natural ribonuclease and that is capable of cleaving RNA molecules is an interesting research subject.[1] The conjugation of such artificial ribonucleases to a nucleic acid would potentially result in a ribonuclease capable of cleaving its target mRNA in a sequence-specific manner. Our study on artificial ribonucleases has resulted in the synthesis of imidazole-derived constructs in combination with or without additional amino, hydroxy, or guanidinium groups.[2–4] Different procedures for derivatization of oligonucleotides have been reviewed recently.[5] However, the procedure of derivatization of polyfunctional molecules is a difficult task.

Recently, we have described a series of monomers for oligonucleotide conjugate synthesis[6,7] using a novel precursor strategy.[8] Using this technique we have prepared a series of oligonucleotide based artificial ribonucleases bearing diimidazole catalytic group.[9] We believe that this strategy in combination with

Accepted 2 May 2005.
This work was supported by the Russian Fund for Fundamental Research, Grants No 02-04-48664a, 03-04-48562a, 04-04-48566a, and 04-03-32490a. Authors are grateful to Dr. D. Kolpashchikov (Columbia University, New York) for fruitful discussion and Eric Green (Columbia University, New York) for careful reading of the manuscript.
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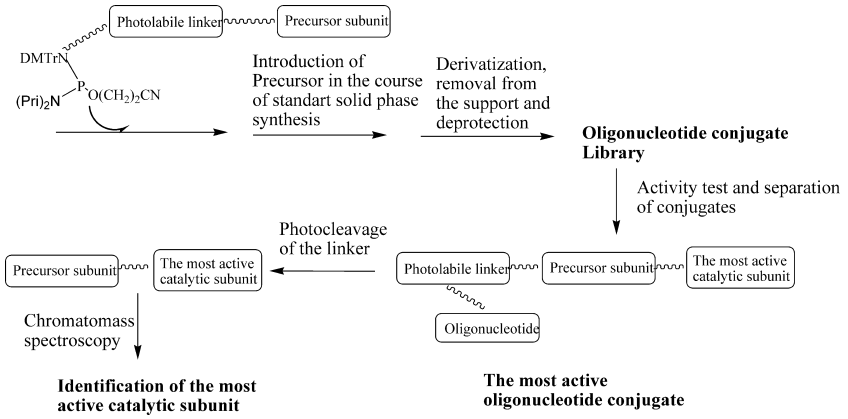

FIGURE 1 General scheme of using a cleavable linker and precursor subunit in optimization of catalytic group structure in oligonucleotide based RNAse mimic library.

elements of combinatorial chemistry may be useful for optimization of catalytic group structure (Figure 1).

For realization of this idea we have combined a photocleavable linker and active precursor group in one molecule. We demonstrate the possibility of such approach by the synthesis and preliminary photolytic study and establishment of the minimal RNAse mimic model structure (Figure 2).

RESULTS AND DISCUSSION

Design of the Photocleavable Linker

In the last decade, special attention has been devoted to linkers potentially suitable for the purposes of combinatorial chemistry: stable under reaction conditions and easily cleavable without damaging biopolymers.[10–13] Well-known *o*-nitrobenzyl and related compounds seem to be the most promising due to their

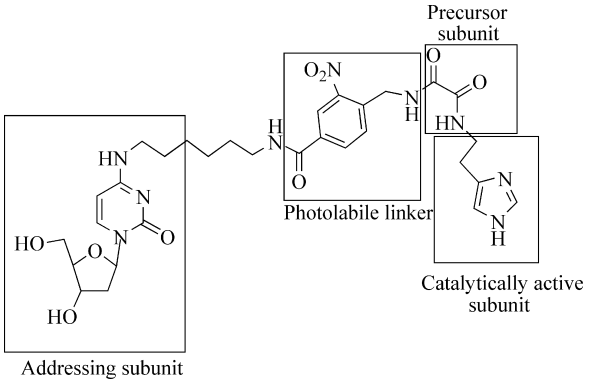

FIGURE 2 RNAse mimic model.

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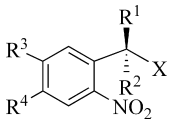

FIGURE 3 Photolabile linker based on the *o*-nitrobenzyl fragment.

ability to release a linked (or "caged") residue upon UV irradiation, with minimal or no modifications.[14]

Some constructs based on the *o*-nitrobenzyl fragment have a chiral carbon atom in their structure (Figure 3, when $R^1 \neq R^2$). This is not problematic for the purpose of creating an oligomer library, since the diastereomeric linker is absent from the target compounds.[13,15–17] In some cases, the authors have assumed that a racemic mixture of linker did not effect the functioning of the final compounds they obtained.[18,19] Since our target compounds will include the photocleavable linker, as well as a library, we must assume that different isomers of the linker could affect the RNA cleaving effectiveness of the RNAse mimic. Thus, the stereochemistry of the conjugate (Figure 2) is very important, and racemic mixtures in any part of the molecule have to be avoided. Hence, R^1 must be equal R^2 (Figure 3); both may be a hydrogen atom for simplicity and diminution of any rotational hindrance at this site.

Since most biopolymers do not absorb light in the range 300–400 nm, long wavelength UV light is less damaging. According to the literature *o*-nitroveratryl (when $R^3 = R^4 = OCH_3$, Figure 3) and related compounds are more sensitive to UV light at wavelengths greater than 300 nm (ε_{360} 5000 $M^{-1}cm^{-1}$) than *o*-nitrobenzyl compounds (ε_{350} 500 $M^{-1}cm^{-1}$)[20] and display faster photolytic kinetics.[21] To combine the advantages of a long wavelength UV absorbance band and the utility of a carboxyl functional group, a number of studies synthesized and used *o*-nitroveratrole based compounds, where R^3 is $-OCH_3$ and R^4 is $-O(CH_2)_nCOOH$.[12,13,21–24] While the *o*-nitroveratryl derivatives have a larger absorbance than *o*-nitrobenzyl within the range of 300–400 nm, quantum yield of the photolytic cleavage in this case is less, resulting in a less than tenfold improvement in sensitivity for long wavelength UV light.[20,25] As shown previously, the rate of photolysis depends on the nature of the bond broken and the reaction media.[14] Also, the introduction of additional elements such as methoxy and alkoxy groups in the linker structure complicates the synthesis. Keeping in mind that the possible damaging of the oligonucleotide addressing part of the target molecule (Figures 1, 2) during UV irradiation is not important for our purposes (analysis of the catalytic subunits of RNAse mimics libraries), we chose to construct a linker (Figure 3) where $R^1 = R^2 = R^3 = H$ and $R^4 = COOH$.

Synthesis of the RNAse Mimic Model

In order to verify the applicability of the designed 4-(aminomethyl)-3-nitrobenzoic acid as a photocleavable linker for analysis of oligonucleotide RNAse

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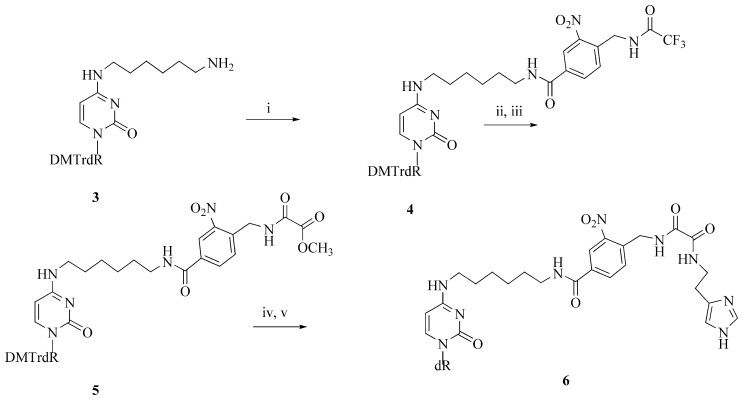

SCHEME 1 Synthesis of the model RNAse mimic. i) Dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, compound **2**, acetone; ii) NH_3/H_2O; iii) dimethyl oxalate, TEA, MeOH; iv) histamine·2HCl, TEA, MeOH; v) $HOAc/H_2O$.

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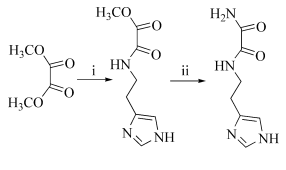
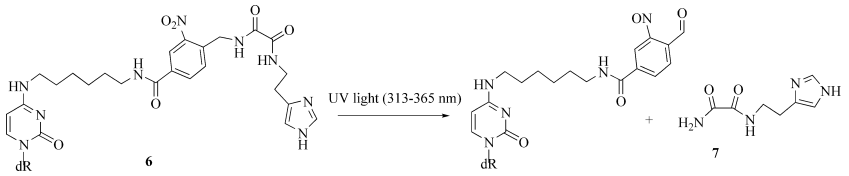
mimics, we synthesized a model compound consisting of addressing, photocleavable, precursor and catalytic subunits (Figure 2). First, the protected photocleavable linker was synthesized. Previously, linkers based on 4-(aminomethyl)-3-nitrobenzoic acid were used for solid-phase peptide synthesis.[17,26] In both cases nitration was performed on 4-(bromomethyl)benzoic acid. We decided to use commercially available 4-(aminomethyl)benzoic acid as a starting material. An amine group of this acid was blocked with trifluoroacetyl residue. The nitration of 4-(trifluoroacetamidomethyl)benzoic acid **1** with 68% nitric acid in sulfuric acid solution at 0°C afforded 3-nitro-4-(trifluoroacetamidomethyl)benzoic acid **2** with a 75% yield. The protected amino acid **2** was converted to a 1-hydroxybenzotriazole ester and coupled to the 4-*N*-(6-aminohexyl)-5′-*O*-(4,4′-dimetoxytrityl)-2′-deoxycytidine **3** (Scheme 1) to give the aminoacylated derivative **4** (Scheme 2). Compound **3** was prepared similarly to a 2′-deoxycytidine derivative with a three methylene group linker.[6] Compound **4** was deblocked by ammonia treatment and reacted with dimethyl oxalate to give **5** (Scheme 1). At this stage it would be possible to convert the 5′-DMTr-2′-deoxycytidine derivative **5** to a 3′-phosphoramidite by a standard procedure;[6] the resulting monomer could be used in oligodeoxyribonucleotide synthesis to obtain oligonucleotide containing libraries with a photocleavable tether using the precursor strategy (Figure 1). This is a work in progress,



SCHEME 2 Synthesis of the amidooxalhistamide **7**. i) Histamine 2HCl, TEA, MeOH; ii) NH_3/MeOH.



SCHEME 3 Products of the photolytic cleavage of the RNAse mimic model **6**.

and in this article we decide to limit an addressing part of the RNAse mimic model to monomer (Figure 2); so we do not include results concerning the oligonucleotide synthesis. Imidazole containing constructs are widely used as small RNAse mimics.[27] It has been shown previously that the simplest artificial RNAse includes histamine residue.[28] Therefore, we treated compound **5** with histamine to introduce an imidazole containing residue as a model for the catalytic group. Finally, we treated the crude product with acetic acid to deblock the final conjugate (Scheme 1). Compound **6** was purified by reverse-phase chromatography, and characterized by NMR and mass spectroscopy.

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Photolysis of the Model RNAse Mimic

When **6** was subjected to long wavelength UV light (313–365 nm), the starting compound disappeared with a $\tau_{1/2}$ of 5.0 min. The photofragmentation reaction was monitored using HPLC (data not shown). These data are in agreement with the half-life for photolabile linker published earlier.[18] Based on the well-known mechanism of photolytic cleavage of *o*-nitrobenzyl and related systems,[14] we expected the formation of the amidooxalhistamide **7** (Scheme 2) as an imidazole containing photolysis product. In order to confirm this we synthesized compound **7** separately (Scheme 3) to use as a standard in the search for product **7**. The molar absorption coefficient ε_{260} of compound **7** was found to be rather low (334 $M^{-1}cm^{-1}$). Using multiwave detection and calculations of the spectral ratios we succeeded in identifying the amidooxalhistamide in HPLC profiles of irradiated samples of **6**, using authentic sample of **7** as a reference substance. In a separate experiment compound **7** was proved to be stable during irradiation (data not shown). The liberated product was separated from photolysis reaction mixture by HPLC and was subjected to NMR, UV, and mass spectra analysis. The spectral data were found to be identical to amidooxalhistamide **7**.

EXPERIMENTAL SECTION

General

The following reagents and equipment were used: N^4-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (ChemGenes Corporation, Wilmington, MA, USA), 4-(Aminomethyl)benzoic acid and 1,5,7-triazabicyclo[4.4.0]dec-5-en (Fluka

Chemica, Buchs, Switzerland), histamine dihydrochloride (Sigma, St. Louis, MO, USA). All other reagents were from Aldrich, WI, USA. Organic solvents were dried and purified by standard procedures. NMR spectra were acquired on Brucker AM-400 and AC-200 spectrometers in approapriate deuterated solvents at 30°C. Chemical shifts (δ) are reported in ppm relative to TMS signals (internal standard, in the case of ^{19}F C_6F_6 was used as an external standard). Coupling constants J are reported in Hertz. Mass spectra were run on a Finnigan-MAT-8200 (EI, 70 eV) (Thermo Electron Corp., Somerset, NJ, USA) and Reflex III (MALDI) (Brucker, Bremen, Germany). Dihydroxybenzoic acid was used as a matrix in MALDI spectra. Melting points were measured on a Kofler hot stage (VEB Analytik). HPLC was performed on a Milichrom-4 (Econova, Novosibirsk, Russia). A 2 × 60 mm column packed with Nucleosil C_{18} (5 μm), having a gradient elution of acetonitrile in 0.03 M $LiClO_4$ (0 to 50%) over 25 min, with an elution rate 0.1 mL per min, was used for the analysis. Thin layer chromatography was performed using Alufolien Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany) in appropriate solvent mixtures and visualized by UV irradiation, ninhydrin (amine groups), cystein/sulfuric acid (nucleoside) or *p*-diazobezenesulfonic acid (imidazole residue).

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4-(Trifluoroacetamidomethyl)benzoic acid (1). 4-(Aminomethyl)benzoic acid (3 g, 20 mmol) was suspended in a mixture of methanol (20 mL) and triethylamine (4.2 mL, 30 mmol). Ethyl trifluoroacetate (4.2 mL, 36 mmol) was added dropwise to the suspension and the reaction mixture was stirred overnight. After the reaction was complete the clear solution was evaporated. The residue was treated with boiling water (70 mL). The clear solution was chilled to room temperature and acidified to pH 3 with trifluoroacetic acid. The acidified solution was cooled in an ice bath, and afforded 4-(trifluoroacetamidomethyl)-benzoic acid **1** as a white powder (4.23 g, 84%) after filtration and drying in a vacuum. R_f 0.44 (CH_2Cl_2:MeOH 9:1); m.p. 206–208°C; ^1H NMR (200 MHz, [D_6]-DMSO): δ 4.46 (2H, d, J 6.0, –CH_2NH–), 7.39 (2H, d, J 8.0, *m*-C*H*–), 7.93 (2H, d, J 8.0, *o*-C*H*–), 10.03 (1H, br t, J 6.0, –N*H*–), 12.67 (1H, br s, –O*H*); ^{13}C NMR (50 MHz, [D_6]-DMSO): δ 42.30, 115.89 (q, $J_{C,F}$ 287), 127.27, 129.44, 129.83, 142.28, 156.43 (q, $J_{C,F}$ 37), 166.91; ^{19}F NMR (188 MHz, [D_6]-DMSO): δ 90.52; EI-MS: *m/z* (%) 247 (91) [M^+], 202 (100) [M^+–COOH]; elemental analysis calcd for $C_{10}H_8F_3NO_3$ (247.17): C 48.59, H 3.26, F 23.06, N 5.67; found, C 48.86, H 3.15, F 22.92, N 5.46.

3-Nitro-4-(trifluoroacetamidomethyl)benzoic acid (2). (Trifluoroacetamidomethyl)benzoic acid **1** (4 g, 16 mmol) was dissolved in sulfuric acid (60 mL) and the solution was chilled in an ice bath. A mixture of nitric acid (68%, 1.6 mL) and sulfuric acid (98%, 1.92 mL) was added to the solution dropwise with stirring over a period of half an hour. The ice was removed and the reaction mixture was left to warm to room temperature. After 2 h the solution was poured onto 500 g of ice. The resultant slurry was filtered to give a white

powder. The solid was washed several times with water and recystallized from ethanol/water to afford the nitrated trifluoroacetamide **2** (4.0 g, 86%). R_f 0.28 (CH_2Cl_2:MeOH 9:1); m.p. 196–198°C; ^{1}H NMR (400 MHz, [D_6]-DMSO): δ 4.78 (1H, d, *J* 5.6, –C*H*$_2$NH–), 7.66 (1H, d, *J* 8.0, –C*H*^{5}–), 8.25 (1H, dd, *J* 8.0, 1.6, –C*H*^{6}–), 8.48 (1H, d, *J* 1.6,–C*H*^{2}–), 9.87 (1H, br t, *J* 5.6, –N*H*CO–); ^{13}C NMR (100 MHz, [D_6]- DMSO): δ 40.13, 115.54 (q, $J_{C,F}$ 286), 124.86, 130.01, 131.46, 133.45, 135.71, 147.86, 156.51 (q, $J_{C,F}$ 37), 164.81; ^{19}F NMR (376 MHz, [D_6]- DMSO): δ 89.64; MS (MALDI) *m/z* 291.29 [M-H], 246.14 [M-NO_2], 178.82 [$C_8H_5NO_4$] (3-nitroso-4-formylbenzoic acid, the product of the photolysis of the compound **2**); elemental analysis calcd for $C_{10}H_7F_3N_2O_5$ (292.17): C 41.11, H 2.41, F 19.51, N 9.59; found, C 40.95, H 2.32, F 19.43, N 9.33.

4-*N*-(6-Aminohexyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (3). 2′-Deoxycytine derivative **3** (Scheme 1) was prepared from 5′-*O*-DMTr-2′-dC^{Bz} following a published procedure[6] using 1,6-diaminohexane instead of 1,3-diaminopropane. Yield was 40%. R_f 0.36 (CH_2CL_2:MeOH:TEA 7:2:1); ^{1}H NMR (200 MHz, [D_6]-acetone plus several drops of [D_5]-pyridine) δ 1.30–1.43 (4H, m, 2x-C*H*$_2$–), 1.48–1.63 (4H, m, 2x-C*H*$_2$–), 2.11–2.22 (1H, m, H2′), 2.29–2.40 (1H, m, H2″), 3.14 (2H, t, *J* 6.5, –C*H*$_2$NH$_2$), 3.28–3.42 (4H, m, H5′,5″,–NHC*H*$_2$–), 3.75 (6H, s, 2xC*H*$_3$O–), 4.01–4.10 (1H, m, H4′), 4.46–4.59 (1H, m, H3′), 5.63 (1H, d, *J* 7.1, H5), 6.35 (1H, app. t, *J* 6.1, H1′), 6.85 (4H, d, *J* 8.6, DMTr), 7.15–7.50 (9H, m, DMTr), 7.73 (1H, d, *J* 7.1, H6).

4-*N*-{6-[3-Nitro-4-(trifluoroacetamidomethyl)]benzamidohexyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (4). 3-Nitro-4-(trifluoroacetamidomethyl)benzoic acid **2** (Scheme 1) (93 mg, 0.3 mmol), dicyclohexylcarbodiimide (80 mg, 0.4 mmol), and 1-hydroxybenzotriazole (47 mg, 0.35 mmol) were dissolved in acetone (2 mL) and the reaction mixture was stirred for 1 h. Compound **3** (157 mg, 0.25 mmol) and TEA (0.07 mL, 0.5 mmol) were then added. After 2 h of stirring, the reaction mixture was evaporated, and the residue was suspended in methylene chloride and filtered. The filtrate was applied to a column packed with silica gel (50 mL). Elution was performed with a linear gradient of methanol in methylene chloride (0–10%) containing 0.1% of pyridine. Appropriate fractions were pooled and evaporated to give 140 mg (0.155 mmol, 62%) of derivative **4** as white foam. R_f 0.27 (CH_2Cl_2:MeOH 9:1); ^{1}H NMR (200 MHz, [D_6]-acetone): δ 1.28–1.49 (4H, m, 2x-C*H*$_2$–), 1.49–1.72 (4H, m, 2x-C*H*$_2$–), 2.12–2.22 (1H, m, H2′), 2.29–2.40 (1H, m, H2″), 3.31–3.50 (6H, m, H5′,5″, –NHC*H*–, –C*H*$_2$NHCO–), 3.78 (6H, s, 2xC*H*$_3$O–), 3.97–4.09 (1H, m, H4′), 4.42–4.58 (1H, m, H3′), 4.89 (2H, s, Ar-C*H*$_2$NH–), 5.60 (1H, d, *J* 7.5, H5), 6.35 (1H, app. t, *J* 6.0, H1′), 6.85 (4H, dt, *J* 8.6, 2.7, DMTr), 7.18–7.52 (9H, m, DMTr), 7.76 (1H, d, *J* 8.0, –C*H*^{5}-(Ar)), 7.87 (1H, d, *J* 7.5, H6), 8.30 (1H, dd, *J* 8.0, 1.6, –C*H*^{6}–(Ar)), 8.52 (2H, m, 2x-N*H*CO–), 8.65 (1H, d, *J* 1.6, –C*H*^{2}–(Ar)).

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4-*N*-{6-[3-Nitro-4-(methoxyoxalamidomethyl)]benzamidohexyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (5). Compound **4** (Scheme 1) (140 mg, 0.155 mmol) was dissolved in 5 mL of 1,4-dioxane. Concentrated ammonia (5 mL) was added to the solution and the reaction mixture was stirred overnight. The solution was evaporated; the residue was dissolved in 25 mL of methylene chloride and washed several times with water. The organic layer was dried with Na_2SO_4 and concentrated to 5 mL. The nucleoside derivative was precipitated with a tenfold volume of hexane to give 4-*N*-{6-[3-nitro-4-(aminomethyl)]benzamidohexyl}-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (120 mg, 93%) as a white solid. R_f 0.1 (CH_2Cl_2:MeOH 8:2); ^{1}H NMR (200 MHz, [D_6]-acetone plus several drops of [D_5]-pyridine): δ 1.30–1.50 (4H, m, 2x-CH_2–), 1.51–1.77 (4H, m, 2x-CH_2–), 2.09–2.28 (1H, m, H2′), 2.32–2.50 (1H, m, H2″), 3.28–3.51 (6H, m, H5′,5″,-NHCH–,–CH_2NHCO–), 3.77 (6H, s, 2xCH_3O–), 4.00–4.15 (1H, m, H4′), 4.48–4.60 (1H, m, H3′), 4.71 (2H, s, Ar-CH_2NH_2–), 5.62 (1H, d, *J* 7.5, H5), 6.36 (1H, app. t, *J* 6.0, H1′), 6.85 (4H, dt, *J* 8.6, 2.7, DMTr), 7.18–7.52 (9H, m, DMTr), 7.77 (1H, d, –CH^5- (Ar)), 7.91 (1H, d, *J* 7.5, H6), 8.27 (1H, dd, *J* 8.0, 1.6, –CH^6– (Ar)), 8.56 (1H, br t, *J* 5.0, –NHCO–), 8.58 (1H, d, *J* 1.6, –CH^2–(Ar)). The nucleoside derivative with the free amino group (120 mg, 0.145 mmol) was dissolved in the mixture of methanol (1.5 mL) and TEA (0.15 mL) and the solution was added to solution of dimethyl oxalate (85 mg, 0.7 mmol) in 2 mL of methanol over a 6-h period. The reaction mixture was stirred overnight, and then evaporated. The residue was dissolved in methylene chloride (5 mL) and the solution was applied to a column packed with silica gel (50 mL). Elution was performed with a linear gradient of methanol in methylene chloride (0–10%) containing 0.1% of pyridine. Appropriate fractions were pooled and evaporated to give derivative **5** (Scheme 1) (90 mg, 69%) as a white foam. R_f 0.25 (CH_2Cl_2:MeOH 9:1); ^{1}H NMR (200 MHz, [D_6]-acetone): δ 1.35–1.50 (4H, m, 2x-CH_2–), 1.51–1.76 (4H, m, 2x-CH_2–), 2.10–2.26 (1H, m, H2′), 2.27–2.43 (1H, m, H2″), 3.30–3.49 (6H, m, H5′,5″, –NHCH–, –CH_2NHCO–), 3.77 (6H, s, 2xCH_3O– (DMTr)), 3.82 (3H, s, –OCH_3(Ox)), 3.98–4.07 (1H, m, H4′), 4.46–4.58 (1H, m, H3′), 4.84 (2H, br s, Ar-CH_2NH–), 5.61 (2H, d, *J* 7.5, H5), 6.29 (1H, app. t, *J* 6.0, H1′), 6.85 (4H, dt, *J* 8.6, 2.7, DMTr), 7.18–7.52 (9H, m, DMTr), 7.71 (9H, d, *J* 8.0, –CH^5–(Ar)), 7.77 (1H, d, *J* 7.5, H6), 8.28 (1H, dd, *J* 8.0, 1.6, –CH^6–(Ar)), 8.54–8.72 (3H, m, –CH^2–(Ar), 2x-NHCO–).

4-*N*-{6-[3-Nitro-4-(2-(4-imidazolyl)ethylamidooxalamidomethyl)]benzamidohexyl}-2′-deoxycytidine (6). Histamine dihydrochloride (54 mg, 0.4 mmol) was dissolved in a mixture of methanol (2 mL) and TEA (0.5 mL). The derivative **5** (Scheme 1) (90 mg, 0.1 mmol) was dissolved in methanol (1 mL), and both solutions were combined. The reaction mixture was stirred overnight, and then evaporated; the residue was dissolved in methylene chloride (10 mL) and washed several times with water. The organic layer was evaporated; its residue was dissolved in 80% aqueous acetic acid (5 mL). After the

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deprotection was complete (1 h) the reaction mixture was evaporated several times with water to remove acetic acid, the residue was dissolved in water (10 mL), the solution was filtered and applied to a column (30 mL) packed with Porasil C_{18} (55–105 μm, Waters, Milford, MA, USA). Elution was performed with a linear gradient of acetonitrile in water (0–50%). Fractions containing the target product were evaporated to dryness to give the title compound **6** (Scheme 1) (20 mg, 30%). ^{1}H NMR (200 M, [D_6]-acetone): δ 1.30–1.48 (4H, m, 2x-C*H*$_2$–), 1.49–1.69 (4H, m, 2x-C*H*$_2$–), 2.10–2.22 (1H, m, H2′), 2.25–2.40 (1H, m, H2″), 2.78 (2H, t, *J* 7.0, -CH$_2$C*H*-Im), 3.40–3.68 (6H, m, –NHC*H*–, 2x-C*H*$_2$NHCO–), 3.70–3.89 (2H, m, H5′,5″), 4.21–4.41 (2H, m, H3′,H4′), 4.69–4.79 (2H, m, Ar-C*H*$_2$NH–), 5.74 (1H, d, *J* 7.5, H5), 6.24 (1H, app. t, *J* 6.0, H1′), 6.87 (1H, s, H^5(Im)), 7.61 (1H, s, H^2(Im)), 7.62 (1H, d, *J* 8.0, –C*H*^{5}–(Ar)), 7.80 (1H, d, *J* 7.5, H6), 8.24 (1H, dd, *J* 8.0, 1.6, –C*H*^{6}–(Ar)), 8.56 (1H, d, *J* 1.6, –C*H*^{2}–(Ar)), 8.72–8.86 (2H, m, 2x-N*H*CO–), 9.28 (2H, t, *J* 5.5, –N*H*CO); MS (MALDI) *m/z* 669.97 [M + H].

2-(4-Imidazolyl)ethylamidooxalamide (7). Dimethyl oxalate (236 mg, 2 mmol) was dissolved in methanol (2 mL). Histamine dihydrochloride (331 mg, 1.8 mmol) was dissolved in a mixture of methanol (2 mL) and TEA (0.6 mL), and this solution was added to the dimethyl oxalate over period of 2 h with continuous stirring. The reaction mixture was heated under reflux for 30 min and left overnight at room temperature. The reaction mixture was concentrated twofold and the precipitate was collected by filtration, washed with methanol, and dried under a vacuum. Intermediate methoxyoxalyl-2(4-imidazolyl)ethylamide was treated with 6.6 M NH_3/MeOH (5 mL) for 10 min. The solution was evaporated, and the residue was recrystallized from water to afford the title compound **7** (Scheme 2) (0.150 g, 46%). R_f 0.25 (CH_2Cl_2:MeOH 8:2); m.p. 210–212°C; ^{1}H NMR (400 MHz, [D_6]- DMSO): δ 2.69 (2H, t, *J* 7.0, –C*H*-Im), 3.36 (2H, dt, *J* 7.0, 6.0, –NHC*H*–CH$_2$–), 6.81 (1H, s, H5 (Im)), 7.53 (1H, s, H2 (Im)), 7.78 (1H, s, N*H*$_2$–), 8.05 (1H, s, N*H*$_2$–), 8.75 (1H, t, *J* 6.0, –N*H*CO–), 11.82 (1H, br s, –N*H*-(Im)); ^{13}C NMR (50 MHz, [D_6]-DMSO): δ 26.43, 38.88, 116.13, 116.40, 134.67, 160.05, 162.12; EI-MS *m/z* (%): 182 (7.5) [M^+], 138 (23.3) [M^+-H_2NCO]; elemental analysis calcd. for $C_7H_{10}N_4O_2$ (182.18): C 46.15, H 5.53, N 30.75; found, C 45.86, H 5.48, N 30.46.

Photolysis Conditions

Photocleavage of model RNAse mimic **6** was performed at room temperature using irradiation in the range of 313–365 nm (filters BS and UFS-2) with a high pressure Hg lamp (DRK-120, St. Petersburg, Russia, *W* 0.3 mW/cm^2). The methanolic solution of **7** (5.4×10^{-3} M, 0.05 ml) was diluted tenfold with water and 0.1 mL aliquots were placed under the lamp at a distance of 10 cm. Aliquots were analyzed by the reverse-phase HPLC. The degree of the photolysis was estimated by the disappearance of the starting compound **6**.

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CONCLUSION

The design and synthesis of the photocleavable *o*-nitrobenzyl based linker suitable for the synthesis and analysis of oligonucleotide containing combinatorial libraries were successful. The simplest RNAse mimic model containing the addressing, photocleavable, precursor and catalytic subunits was designed and synthesized. Upon irradiation (313–365 nm), it released the catalytic subunit combined with the precursor residue which was identified in the course of chromatographic analysis.

In the future we plan to construct a chimeric oligonucleotide that includes both an addressing part of RNAse mimic and an RNA target. It will also use a library of catalytically active constructs joined to the addressing part of the molecule through the photocleavable linker described in this paper. Active in RNA cleavage chimeras will be separated from inactive constructs by gel electrophoresis. After selecting the most active constructs, the catalytic subunits would be cleaved from the rest of the molecule and analyzed by chromatomass spectroscopy.

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