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PAPER

Diamondoid-modified DNA†

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We prepared novel C5-modified triphosphates and phosphoramidites with a diamondoid functionally linked to the nucleobase. Using primer extension experiments with different length templates we investigated whether the modified triphosphates were enzymatically incorporated into DNA and whether they were further extended. We found that all three modified nucleotides can be incorporated into DNA using a single-nucleotide incorporation experiment, but only partially using two templates that demand for multiple incorporation of the modified nucleotides. The modified phosphoramidites were introduced into oligonucleotides utilizing DNA synthesizer technology. The occurring oligonucleotide structures were examined by circular dichroism (CD) and melting temperature (T_m) measurements and were found to adapt similar helix conformations as their unmodified counterparts.

Introduction

Deoxyribonucleic acid (DNA) represents the complete genetic database of nature and thus contains all information necessary for synthesis of ribonucleic acid (RNA) and proteins. These are required for the development of a biological organism and the metabolism in the cells. Since the discovery of DNA it has been in constant focus of molecular biology and biochemical research. There is a growing interest in the synthesis of functionalized DNA, and an important aspect is that by modifying the structure of the double stranded DNA it must not significantly compromise duplex formation. Most appropriate for functionalization is the part of the nucleobase that is not involved in hydrogen bonds of Watson–Crick base pairing.[1–3] In addition, when utilizing sterically demanding moieties a sufficiently long linker should be used to reduce the interactions between DNA and the added structural moiety. Particularly attractive, sterically highly demanding building blocks are the so-called diamondoids (nanodiamonds), which are a member of the nanoscale carbon materials family, consisting of face-fused carbon cage (adamantane) repeating units that are superimposable on the diamond lattice. Their terminal bonds are saturated by hydrogen, leading to a unique molecular hierarchy of the form $C_{4n+6}H_{4n+12}$, where $n = 1, 2, 3, \ldots$ specifies the polymantane order (Fig. 1).[4–6] Diamondoids display a variety of geometries, offering a large tool kit of atomically-perfect building blocks of different shapes and sizes. Following isolation from petroleum deposits,[7] the diamondoids can be selectively derivatized by substituting various functional groups for surface hydrogens,[8] and their derivatives have found widespread applications from materials science[9] to medical applications.[10]

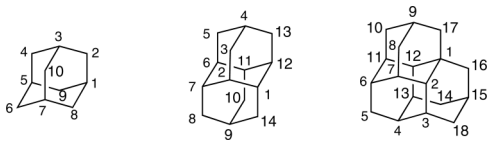

Fig. 1 Structures of the selected diamondoids and their nomenclature: a) adamantane, b) diamantane, and c) triamantane.

Here we show, for the first time, that diamondoids (Fig. 1) can be introduced as a lipophilic and bulky functional group at the C5-position of the pyrimidine moiety and then incorporated into DNA by chemical and enzymatic means. It has been shown that modifications at this position do not significantly interfere with Watson–Crick base pairing.[11–20] Here we report the synthesis of diamondoid-modified triphosphates and phosphoramidites (Fig. 2). We found that these nucleotides can be incorporated into

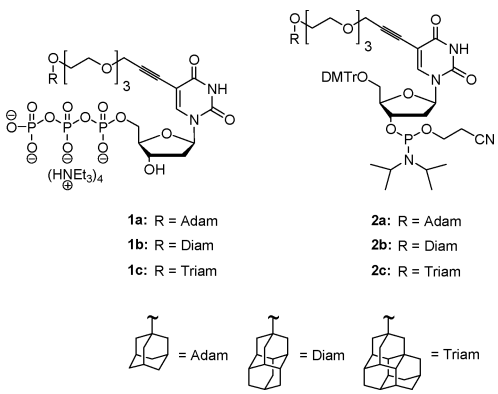

Fig. 2 Diamondoid modified triphosphates and phosphoramidites.

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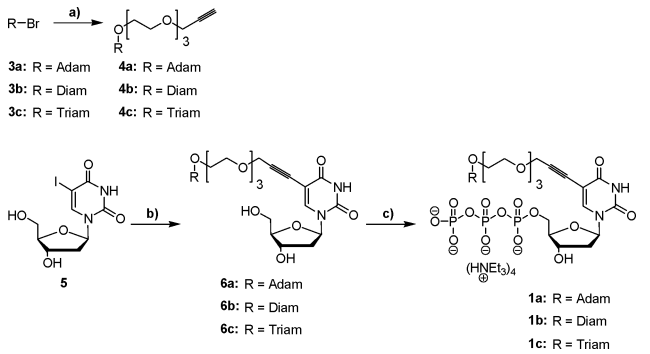
DNA by DNA polymerases and also by automated solid phase DNA synthesis. The conformation of the resulting diamondoid-modified DNA was investigated by thermal denaturation studies (T_m measurements) as well as circular dichroism (CD) spectroscopy.

Results and discussion

Synthesis of modified triphosphates

In order to place the bulky diamondoid modification away from the nucleobase we choose a triethylene glycol linkage (Fig. 2) that has been shown to be accepted by DNA polymerases.[16] First we planned the synthesis of 1-*O*-diamantoidyl-4-*O*-propargyl triethylene glycol **4**. For this purpose, the bromides **3a,b,c**[21–23] were prepared and heated in the presence of triethylamine with 1-*O*-propargyl triethylene glycol[24,25] for 5 h at 180 °C and converted into 1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol **4a,b,c**. The compounds **4a,b,c** were then coupled to 5-iodo-2′-deoxyuridine **5** by using a standard protocol for the Sonogashira reaction[16,26–28] to obtain nucleosides **6a,b,c**. These nucleosides were then converted into the corresponding triphosphates **1a,b,c** by using an appropriate method for phosphorylation[29–31] (Scheme 1).



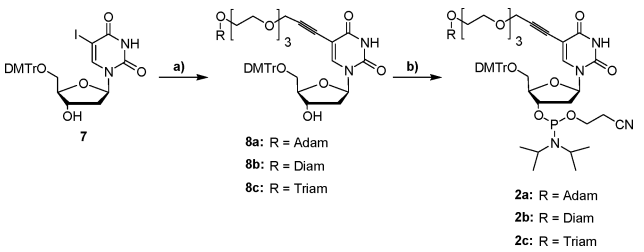
Scheme 1 a) 1-*O*-propargyl triethylene glycol, Et_3N, 180 °C, 5 h, **4a**: 83%, **4b**: 76%, **4c**: 79%; b) CuI, $[Pd(PPh_3)_4]$, Et_3N, 1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol **4**, DMF, rt, 24 h, **6a**: 75%, **6b**: 75%, **6c**: 79%; c) proton sponge (1,8-bis(dimethylamino)naphthalene), $POCl_3$, $PO(OMe)_3$, 0 °C, then $(Bu_3NH)_2H_2P_2O_7$, nBu_3N, then triethylammonium bicarbonate (TEAB) buffer, **1a**: 6%, **1b**: 5%, **1c**: 5%.

Synthesis of modified phosphoramidites

The procedure for the synthesis of C5-modified 2′-deoxyuridine-3′-*O*-phosphoramidites **2** was similar to that for the triphosphates. 5-Iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-uridine **7** was prepared according to literature protocols[32,33] and was coupled to **4a,b,c** by using the Sonogashira reaction[26–28] to create nucleosides **8a,b,c**. The reactions were conducted in a microwave leading to higher yields compared to reactions performed without microwave assistance. The compounds **8a,b,c** were then converted into the corresponding phosphoramidites **2a,b,c** using a standard method[34–36] (Scheme 2). The phosphoramidites **2** were purified rapidly after reaction and stored under argon atmosphere at –20 °C.

Incorporation of diamondoid-functionalised nucleotides using primer extension

The primer extension reactions should provide information whether DNA polymerases are able to incorporate the modified



Scheme 2 a) CuI, $[Pd(PPh_3)_4]$, Et_3N, 1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol **4**, DMF, in microwave, 50 °C, 20 min, **8a**: 66%, **8b**: 70%, **8c**: 63%; b) *N*-ethyldiisopropylamine, 2-cyanoethyl-*N*,*N*-diisopropylchloro-phosphoramidite, CH_2Cl_2, 0 °C 30 min, then rt, 3 h, **2a**: 70%, **2b**: 75%, **2c**: 80%.

triphosphates **1** into a growing DNA strand by substitution of the natural counterpart. We carried out primer extension experiments using a 5′-^{32}P-labeled 23-nucleotide primer and several templates. The reactions were analysed by denaturing polyacrylamide gel (PAGE). Visualisation was performed by phosphorimaging.

First we investigated a 35-nucleotide template bearing a single **A** residue at position 27 calling for incorporation of a thymidine analogue. We used *KlenTaq* DNA-polymerase, N-terminally shortened form of Taq DNA polymerase.[37] Lacking dTTP *KlenTaq* DNA polymerase wild-type incorporated a mismatched nucleotide opposite position 27 and then paused.

Reactions including all four natural dNTPs gave rise to full-length products. Full-length product was also observed when natural dTTP was replaced by one of the modified triphosphates **1** (Fig. 3a). In the following experiments we used two different templates (69 nucleotides) to code for a modified dTTP every fourth and second position, respectively. For template calling for a modified nucleotide in every fourth position we found that the DNA polymerase was able to form full-length products in the presence of three natural dNTPs and each of the three modified dTTPs **1** (Fig. 3b). Upon substitution of natural TTP by **1a,b,c** the full-length DNA products migrated more slowly in the gel in

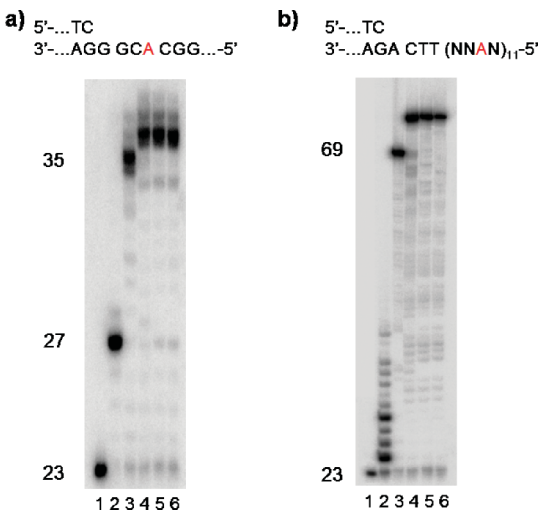


Fig. 3 The primer extension experiments by *KlenTaq* DNA polymerase. For a) and b) Lanes 1: 5′-^{32}P-labeled 23-nucleotide primer strand; lanes 2: primer template complex including dATP, dCTP, dGTP; lanes 3: same as lanes 1 including TTP; lanes 4: same as lanes 1 including **1a**; lanes 5: same as lanes 1 including **1b**; lanes 6: same as lanes 1 including **1c**.

Table 1 Overview of the synthesized oligonucleotides[a]

		Molar masses	
Name	Sequence	Calculated molar mass/(g mol^{-1})	Found molar mass/(g mol^{-1})
ON_Ref	5′-GGT CTT AGC TAT-3′	3751.5	3750.6
ON_Com	5′-ATA GCT AAG ACC-3′	3638.5	3637.4
ON_Adam	5′-GGT CTT* AGC TAT-3′	3957.9	3957.2
ON_Diam	5′-GGT CTT* AGC TAT-3′	4010.0	4008.1
ON_Triam	5′-GGT CTT* AGC TAT-3′	4062.1	4060.3

[a] The oligonucleotides were prepared on a DNA synthesizer. The modifications of the oligonucleotides were inserted using **2a**, **2b** and **2c**. The single strands ON1_Adam, ON1_Diam and ON1_Triam contain the modification of adamantane, diamantane and triamantane.

comparison with the unmodified full-length product (Fig. 3a, b). We suppose that this property is based on the increased steric demand and higher molecular weight of the modified entities; similar effects have been reported before.[13] For the template calling for a modified nucleotide in every second position we found no full-length products and only fragments (data not shown) indicating that the modifications prevent further modification density.

Oligonucleotide synthesis

In order to investigate the duplex properties of diamondoid-modified DNA, the phosphoramidites **2a,b,c** were used to synthesize modified oligonucleotides using a DNA synthesizer.[38,39] The unmodified oligonucleotide[40] (ON_Ref) was used as reference and the oligonucleotide[40] (ON_Com) as the complementary strand. The oligonucleotides ON_Adam, ON_Diam and ON_Triam contained an adamantane-, diamantane-, and triamantane-modified nucleotide. The oligonucleotides were prepared using standard methods.[41–47] An overview of the synthesized oligomers is shown in Table 1.

Circular dichroism (CD) and melting temperature (T_m) measurements

The synthesized oligonucleotides were purified by preparative PAGE and further characterized by thermal denaturation studies (melting temperature (T_m) measurements) as well as by CD spectroscopy. To determine the influence of the modifications on the duplex DNA conformation, CD spectra were measured of the respective modified strand hybridized to the complementary standard ON_Com.

The recorded CD spectra (Fig. 4) showed the characteristic curve of a B-DNA with a positive maximum at about 275 nm and a minimum at about 245 nm for all duplexes. In comparison with the unmodified oligonucleotides the curves of the modified are very much alike. This indicates that the modifications of the investigated diamandoid-modified thymidines are on one hand well accommodated in the major groove, and on the other hand flexible enough without interfering with the overall B-DNA conformation.

The T_m measurements (Fig. 5) indicate duplex stability of the modified oligonucleotides. T_m values of the modified double strands are lower than for the corresponding unmodified DNA strand (54.7 °C). The measured T_m values of adamantane, diamantane, and triamantane modified duplexes are 4.4, 4.9, and 5.2 °C lower, respectively. The decrease of the T_m values indicate a minor destabilization of these modified double strands.

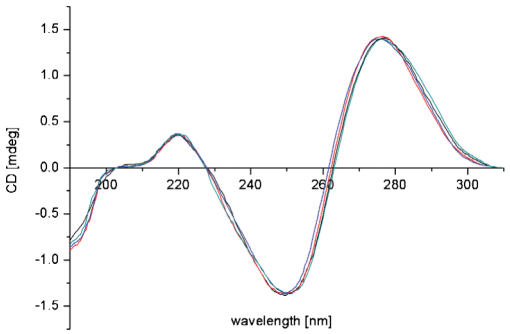


Fig. 4 CD-spectra of unmodified DNA (—) and adamantane modified DNA (——), diadamantane modified DNA (——) and triadamantane modified DNA (——). The CD spectra show the characteristic profile of a B-DNA with a positive maximum at about 275 nm and a minimum at about 250 nm. Compared to the unmodified oligonucleotides the curve of the modified ones do not show significant differences.

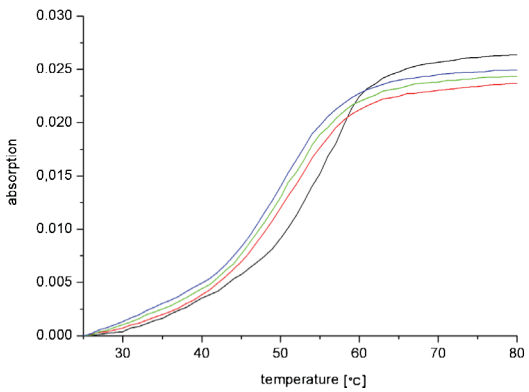


Fig. 5 Melting temperature curves of unmodified DNA (—), adamantane modified DNA (——), diamantane modified DNA (——) and triamantane modified DNA (——). The observed Tm for the unmodified 12 nt DNA was 54.7 °C. The three modified DNA adamantane, diamantane and triamantane show Tm values of 50.3 °C, 49.8 °C and 49.5 °C, respectively.

Conclusions

A straightforward synthesis of diamondoid-modified thymidines was developed. The corresponding triphosphates **1a,b,c** are recognized as substrates for incorporation into the growing DNA strand. Enzymatic synthesis of DNA strands that contain modifications at every fourth position was feasible as well. The corresponding phosphoramidites **2a,b,c** were assembled into oligonucleotides using a DNA synthesizer. CD spectroscopy and melting temperature (T_m) measurements indicate that the

B-conformation of DNA double helix is tolerated by the diamondoid-modified nucleotides, and the decrease of the T_m values show only slight destabilization of the duplex by these modifications. Hence, diamondoid-modified DNA can be utilized to exploit some of the attractive features of these diamond-like molecules by their targeted assembly. Such features include *inter alia* pronounced electron emission.[9]

Experimental section

General

All reagents are commercially available and were used without further purification. Anhydrous solvents were obtained from Sigma–Aldrich, stored over molecular sieves and used without further purification. All synthetic reactions were performed under inert atmosphere. 5-Iodo-2′-deoxyuridine was purchased from Carbosynth. NMR spectra were recorded using Bruker Avance 400 (^{1}H: 400, ^{13}C: 101, ^{32}P: 162 MHz) and DRX 600 (^{1}H: 600, ^{13}C: 151, ^{32}P: 243 MHz) spectrometers. Chemical shifts are given in parts per million, tetramethylsilane was used as internal standard. Electrospray ionization ion trap (ESI-IT) mass spectra were recorded on a Bruker Daltonics Esquire 3000+ in positive or negative mode with a flow rate of 5 mL/min. Flash chromatography was done using Merck silica gel G60 (40–63 μm) and Merck pre-coated plates (silica gel 60 F_{254}) were used for TLC. For medium pressure liquid chromatography (MPLC), a Büchi unit with a Büchi controller C-620, two pumps C-605, a UV monitor C-630 (λ = 254 nm) and fraction collector C-660 was used. For reverse phase chromatography of nucleosides, a D40-RP-18 ready-to-use column (Götec, 25–40 μm) was used. For reverse phase chromatography of nucleotides, a 310-25 LiChroprep® RP-18 ready-to-use column (Merck, 40–63 μm) was used. Purification of triphosphates was performed on a BioLogic DuoFlow System (Bio-Rad Laboratories) with DEAE Sephadex™ A-25 (GE Healthcare Bio-Sciences AB) column using a linear gradient of TEAB-buffer (0.1–1.0 M, pH = 7.5). Desoxynucleotides were purchased from Fermentas, oligonucleotides from Metabion (purified twice by HPLC) and T4 polynucleotide kinase from New England Biolabs. CD spectra was measured on a Jasco J-715 instrument in phosphate-buffer (20 mM K_2HPO_4/KH_2PO_4, pH = 7.0, 1.0 M NaCl) at 25 °C. Melting curves were recorded on a Varian Cary 100 bio UV/Vis Spectrophotometer. The sample (same as for CD spectra) contained 1 × phosphate-buffer (20 mM K_2HPO_4/KH_2PO_4, pH = 7.0, 1.0 M NaCl).

General procedure for 1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol (4a–c)

1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol **4** was synthesized by the reaction of bromodiamondoid **3** (1.0 equiv) and 1-*O*-propargyl triethylene glycol (10 equiv) at 180 °C in the presence of triethylamine (3.0 equiv) for 5 h. After cooling to room temperature ethyl acetate (40 mL) was added. The solution was washed with 2 M hydrochloric acid (2 × 30 mL) and brine (2 × 30 mL). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography.

1-*O*-(1-adamantyl)-4-*O*-propargyl triethylene glycol (4a)

The reaction was carried out with 1-bromo-adamantane **3a** (430 mg, 2.0 mmol), 1-*O*-propargyl triethylene glycol (3.8 g, 20 mmol) and triethylamine (0.80 mL, 6.0 mmol). Purification: silica gel (n-hexane/EtOAc 2 : 1). R_f: 0.43 (n-hexane/EtOAc 2 : 1). Yield: 535 mg (83%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.15 (2H, d, ^4J = 2.4 Hz, OCH_2CCH), 3.67–3.58 (8H, m, OCH_2CH_2OCH_2CH_2O), 3.56–3.50 (4H, m, adam-OCH_2CH_2), 2.40 (1H, t, ^4J = 2.4 Hz, CCH), 2.09 (3H, m, adam-H-3,5,7), 1.69 (6H, m, adam-H-2,8,9), 1.50–1.52 (6H, m, adam-H-4,6,10). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 79.7, 74.5, 72.2, 71.3, 70.6, 70.4, 69.1, 59.3, 58.4, 41.5, 36.5, 30.5. HRMS: *m/z*: calcd for $C_{19}H_{31}O_4^+$ ([M+H]$^+$): 323.2217, found: 323.2208.

1-*O*-(4-diamantyl)-4-*O*-propargyl triethylene glycol (4b)

The reaction was carried out with 4-bromo-diamantane **3b** (219 mg, 0.82 mmol), 1-*O*-propargyl triethylene glycol (1.54 g, 8.2 mmol) and triethylamine (0.35 mL, 2.46 mmol). Purification: silica gel (n-hexane/EtOAc 2 : 1). R_f: 0.49 (n-hexane/EtOAc 2 : 1). Yield: 234 mg (76%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.19 (2H, d, ^4J = 2.4 Hz, OCH_2CCH), 3.69–3.64 (8H, m, OCH_2CH_2OCH_2CH_2O), 3.59–3.57 (4H, m, diam-OCH_2CH_2), 2.42 (1H, t, ^4J = 2.4 Hz, CCH), 1.92 (m, 3H, diam-H-2,6,12), 1.78–1.76 (1H, m, diam-H-9), 1.72–1.69 (15H, m, diam-H-1,3,5,7,8,10,11,13,14). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 79.8, 74.6, 71.7, 71.4, 70.7, 70.5, 69.3, 59.7, 58.5, 41.9, 39.7, 37.3, 36.8, 25.7. HRMS: *m/z*: calcd for $C_{23}H_{35}O_4^+$ ([M+H]$^+$): 375.2530, found: 375.2536.

1-*O*-(9-triamantyl)-4-*O*-propargyl triethylene glycol (4c)

The reaction was carried out with 9-bromo-triamantane **3c** (255 mg, 0.80 mmol), 1-*O*-propargyl triethylene glycol (1.5 g, 8.0 mmol) and triethylamine (0.30 mL, 2.4 mmol). Purification: silica gel (n-hexane/EtOAc 2 : 1). R_f: 0.54 (n-hexane/EtOAc 2 : 1). Yield: 270 mg (79%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.19 (2H, d, ^4J = 2.4 Hz, OCH_2CCH), 3.69–3.65 (8H, m, OCH_2CH_2OCH_2CH_2O), 3.58–3.56 (4H, m, triam-OCH_2CH_2), 2.42 (1H, t, ^4J = 2.4 Hz, CCH), 1.86–1.82 (3H, m, triam-H-7,11,15), 1.70–1.58 (14H, m, triam-H-2,3,4,5,6,12,13,14,16,18), 1.37–1.30 (6H, m, triam-H-8,10,17). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 79.8, 74.6, 72.4, 71.4, 70.7, 70.5, 69.3, 59.7, 58.5, 48.8, 46.1, 45.3, 41.8, 40.3, 38.1, 37.6, 37.5, 35.7, 35.0, 34.5, 27.2. HRMS: *m/z*: calcd for $C_{27}H_{39}O_4^+$ ([M+H]$^+$): 427.2843, found: 427.2839.

General procedure for nucleosides (6a–c)

5-Iodo-2′-deoxyuridine **5** (1.0 equiv) and copper(I) iodide (0.2 equiv) were dissolved in anhydrous DMF. With stirring the corresponding 1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol **4** (3.0 equiv), tetrakis(triphenylphosphine)palladium (0.1 equiv) and triethylamine (3.0 equiv) were added. The mixture was protected from light and stirred at rt for 24 h. The mixture was combined with sat. sodium bicarbonate solution (10 mL) and extracted with dichloromethane (4 × 10 mL). The organic layers were washed with 2 M hydrochloric acid (2 × 10 mL) and with brine (2 × 10 mL), this was dried over magnesium sulfate and the

solvent was evaporated under reduced pressure. The residue was purified by flash chromatography and RP-MPLC.

5-[12-(Adamantyl-1-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine (6a)

The reaction was carried out with 5-iodo-2′-deoxyuridine **5** (229 mg, 0.65 mmol), copper(I) iodide (24.8 mg, 0.13 mmol), 1-*O*-(1-adamantyl)-4-*O*-propargyl triethylene glycol **4a** (420 mg, 1.30 mmol), tetrakis(triphenylphosphine)palladium (75.1 mg, 65 μmol) and triethylamine (0.27 mL, 1.95 mmol) in 5 mL anhydrous DMF. Purification: silica gel (DCM/MeOH 20 : 1) and RP-MPLC (RP-18, 25–40 μm) using a linear gradient of water/acetonitrile (5–100% acetonitrile). The product eluted at 55% acetonitrile to yield 267 mg (75%). R_f: 0.43 (DCM/MeOH 10 : 1). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.29 (1H, s, H-6), 6.18 (1H, t, ^3J = 6.0 Hz, H-1′), 4.52–4.51 (1H, m, H-3′), 4.38–4.30 (2H, m, CCCH_2O), 4.00–3.99 (1H, m, H-4′), 3.90–3.80 (2H, m, H-5′), 3.72–3.64 (8H, m, CH_2OCH_2CH_2OCH_2), 3.60–3.55 (4H, m, adam-OCH_2, CH_2OCH_2CC), 2.44–2.40 (1H, m, H-2′), 2.31–2.25 (1H, m, H-2′), 2.12 (3H, m, Adam-H-3,5,7), 1.72 (6H, m, adam-H-2,8,9), 1.63–1.54 (6H, m, adam-H-4,6,10). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 162.3, 149.7, 144.7, 99.1, 89.3, 87.6, 86.2, 78.0, 72.8, 71.2, 70.5, 70.4, 69.2, 61.6, 59.2, 41.5, 41.1, 36.5, 30.6. HRMS: *m/z*: calcd for $C_{29}H_{41}N_2O_{11}^-$ ([M+HCO_2]$^-$): 593.2716, found: 593.2724.

5-[12-(Diamantyl-4-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine (6b)

The reaction was carried out with 5-iodo-2′-deoxyuridine **5** (35.3 mg, 0.10 mmol), copper(I) iodide (3.8 mg, 20 μmol), 1-*O*-(4-diamantyl)-4-*O*-propargyl triethylene glycol **4b** (74.9 mg, 0.20 mmol), tetrakis(triphenylphosphine)palladium (11.6 mg, 10 μmol) and triethylamine (42 μL, 0.30 mmol) in 1 mL anhydrous DMF. Purification: silica gel (DCM/MeOH 20 : 1) and RP-MPLC (RP-18, 25–40 μm) using a linear gradient of water/acetonitrile (5–100% acetonitrile). The product eluted at 60% acetonitrile to yield 45.1 mg (75%). R_f: 0.51 (DCM/MeOH 10 : 1). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.38 (1H, s, H-6), 6.20 (1H, t, ^3J = 5.2 Hz, H-1′), 4.55–4.54 (1H, m, H-3′), 4.40–4.31 (2H, m, CCCH_2O), 3.99 (1H, m, H-4′), 3.93–3.84 (2H, m, H-5′), 3.69–3.64 (8H, m, CH_2OCH_2CH_2OCH_2), 3.61–3.57 (4H, m, diam-OCH_2, CH_2OCH_2CC), 2.44–2.40 (1H, m, H-2′), 2.32–2.28 (1H, m, H-2′), 1.92 (3H, m, diam-H-2,6,12), 1.78–1.77 (1H, m, diam-H-9), 1.71–1.68 (15H, m, diam-H-1,3,5,7,8,10,11,13,14). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 162.0, 149.6, 144.8, 99.1, 89.3, 87.6, 86.2, 78.1, 72.2, 71.3, 70.6, 70.4, 70.3, 69.2, 61.4, 59.6, 59.3, 41.8, 41.1, 39.7, 37.3, 36.8, 25.7. HRMS: *m/z*: calcd for $C_{33}H_{45}N_2O_{11}^-$ ([M+HCO_2]$^-$): 645.3029, found: 645.3027.

5-[12-(Triamantyl-9-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine (6c)

The reaction was carried out with 5-iodo-2′-deoxyuridine **5** (35.3 mg, 0.10 mmol), copper(I) iodide (3.8 mg, 20 μmol), 1-*O*-(9-triamantyl)-4-*O*-propargyl triethylene glycol **4c** (85.3 mg, 0.20 mmol), tetrakis(triphenylphosphine)palladium (11.6 mg, 10 μmol) and triethylamine (42 μL, 0.30 mmol) in 1 mL anhydrous DMF. Purification: silica gel (DCM/MeOH 20 : 1) and RP-MPLC (RP-18, 25–40 μm) using a linear gradient of water/acetonitrile (5–100% acetonitrile). The product eluted at 64% acetonitrile to yield 51.6 mg (79%). R_f: 0.55 (DCM/MeOH 10 : 1). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42 (1H, s, H-6), 6.21 (1H, t, ^3J = 6.0 Hz, H-1′), 4.58–4.54 (1H, m, H-3′), 4.41–4.31 (2H, m, CCCH_2O), 3.99–3.98 (1H, m, H-4′), 3.95–3.84 (2H, m, H-5′), 3.79–3.66 (8H, m, CH_2OCH_2CH_2OCH_2), 3.62–3.56 (4H, m, triam-OCH_2, CH_2OCH_2CC), 2.46–2.39 (1H, m, H-2′), 2.33–2.27 (1H, m, H-2′), 1.87–1.82 (3H, m, triam-H-7,11,15), 1.71–1.58 (14H, m, triam-H-2,3,4,5,6,12,13,14,16,18), 1.37–1.28 (6H, m, triam-H-8,10,17). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 162.0, 149.6, 144.8, 99.1, 89.2, 87.5, 86.2, 78.2, 73.0, 71.3, 70.6, 70.4, 70.1, 69.2, 61.3, 59.5, 59.3, 48.7, 46.0, 45.3, 41.7, 41.2, 40.2, 38.0, 37.6, 37.4, 35.7, 35.0, 34.4, 27.2. HRMS: *m/z*: calcd for $C_{37}H_{49}N_2O_{11}^-$ ([M+HCO_2]$^-$): 697.3342, found: 697.3342.

General procedure for 5′-*O*-(4,4′-dimethoxytrityl)nucleosides (8a–c)

5-Iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **7** (1.0 equiv) and copper(I) iodide (0.2 equiv) were dissolved in anhydrous DMF. With stirring the corresponding 1-*O*-diamondoidyl-4-*O*-propargyl triethylene glycol **4** (3.0 equiv), tetrakis-(triphenylphosphine)palladium (0.1 equiv) and triethylamine (3.0 equiv) were added. The mixture was stirred in synthesis microwave (Initiator™, Biotage) at 50 °C for 20 min. The mixture was combined with sat. sodium bicarbonate solution (10 mL) and extracted with dichloromethane (4 × 10 mL). The organic layers were washed with 2 M hydrochloric acid (2 × 10 mL) and with brine (2 × 10 mL), this was dried over magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography and RP-MPLC.

5-[12-(Adamantyl-1-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (8a)

The reaction was carried out with 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **7** (197 mg, 0.30 mmol), copper(I) iodide (11.4 mg, 60 μmol), 1-*O*-(1-adamantyl)-4-*O*-propargyl triethylene glycol **4a** (145 mg, 0.50 mmol), tetrakis-(triphenylphosphine)palladium (34.7 mg, 30 μmol) and triethylamine (130 μL 1.5 mmol) in 2 mL anhydrous DMF. Purification: silica gel (EtOAc/n-hexane, 10 : 1 + 1% Et_3N) and RP-MPLC (RP-18, 25–40 μm) using a linear gradient of acetonitrile/water (5–100% acetonitrile). The product eluted at 40% acetonitrile to yield 168.2 mg (66%). R_f: 0.32 (EtOAc/n-hexane, 5 : 1). ^{1}H-NMR (400 MHz, Acetone-d_6): δ 8.08 (1H, s, H-6), 7.53–7.51 (2H, m, Ar–H), 7.41–7.39 (4H, m, Ar–H), 7.36–7.32 (2H, m, Ar–H), 7.26–7.23 (1H, m, Ar–H), 6.92–6.89 (4H, m, Ar–H), 6.28 (1H, t, ^3J = 6.8 Hz, H-1′), 4.61–4.58 (1H, dd, ^3J = 4.4 Hz, ^3J = 7.6 Hz, H-3′), 4.11–4.09 (3H, m, H-4′, CCCH_2O), 3.80 (6H, s, OCH_3), 3.57–3.47 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.42–3.30 (2H, m, H-5′), 2.42–2.39 (2H, dd, ^3J = 4.4 Hz, ^2J = 6.4 Hz, H-2′), 2.09 (3H, m, adam-H-3,5,7), 1.72 (6H, m, adam-H-2,8,9), 1.66–1.58 (6H, m, adam-H-4,6,10). ^{13}C-NMR (101 MHz, Acetone-d_6): δ 161.9, 159.6, 150.3, 146.0, 143.9, 136.9, 136.6, 131.0, 128.9 128.8, 127.7, 114.1, 99.8, 89.9, 87.6, 86.4, 78.6, 72.3, 72.2, 72.0, 71.3, 71.0, 69.8, 64.7, 60.1, 59.3, 55.6, 42.3, 41.8, 37.2, 31.4. HRMS: *m/z*: calcd for $C_{49}H_{57}N_2O_{11}^-$([M – H]$^-$): 849.3968, found: 849.3963.

5-[12-(Diamantyl-4-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (8b)

The reaction was carried out with 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **7** (175 mg, 0.30 mmol), copper(I) iodide (10.0 mg, 54 µmol), 1-*O*-(4-diamantyl)-4-*O*-propargyl triethylene glycol **4b** (150 mg, 0.40 mmol), tetrakis(triphenylphosphine)palladium (31 mg, 27 µmol) and triethylamine (0.1 mL, 0.80 mmol) in 2 mL anhydrous DMF. Purification: silica gel (EtOAc/n-hexane, 10 : 1 + 1% Et_3N) and RP-MPLC (RP-18, 25–40 µm) using a linear gradient of acetonitrile/water (5–100% acetonitrile). The product eluted at 45% acetonitrile to yield 169.1 mg (70%). R_f: 0.36 (EtOAc/n-hexane, 5 : 1). ^{1}H-NMR (400 MHz, Acetone-d_6): δ 8.08 (1H, s, H-6), 7.53–7.51 (2H, m, Ar–H), 7.41–7.39 (4H, m, Ar–H), 7.36–7.32 (2H, m, Ar–H), 7.26–7.23 (1H, m, Ar–H), 6.92–6.89 (4H, m, Ar–H), 6.28 (1H, t, ^3J = 6.8 Hz, H-1′), 4.61–4.58 (1H, dd, ^3J = 4.4 Hz, ^3J = 7.6 Hz, H-3′), 4.12–4.09 (3H, m, H-4′; CCCH_2O), 3.80 (6H, s, OCH_3), 3.57–3.47 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.42–3.31 (2H, m, H-5′), 2.42–2.39 (2H, m, H-2′), 1.92 (3H, m, diam-H-2,6,12), 1.74–1.69 (16H, m, diam-H-1,3,5,7,8,9,10,11,13,14). ^{13}C-NMR (101 MHz, Acetone-d_6): δ 161.9, 159.7, 150.3, 146.0, 143.9, 136.9, 136.6, 131.0, 128.9 128.8, 127.6, 114.1, 99.8, 89.9, 87.6, 86.4, 78.6, 72.4, 72.0, 71.5, 71.3, 71.0, 69.9, 64.7, 60.5, 59.3, 55.6, 42.7, 41.8, 40.5, 38.0, 37.7, 26.6. HRMS: *m/z*: calcd for $C_{53}H_{61}N_2O_{11}^-$ ([M – H]$^-$): 901.4281, found: 901.4282.

5-[12-(Triamantyl-9-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (8c)

The reaction was carried out with 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **7** (185 mg, 0.28 mmol), copper(I) iodide (11.0 mg, 56 µmol), 1-*O*-(9-triamantyl)-4-*O*-propargyl triethylene glycol **4c** (180 mg, 0.42 mmol), tetrakis-(triphenylphosphine)palladium (33 mg, 28 µmol) and triethylamine (0.12 mL, 0.84 mmol) in 2 mL anhydrous DMF. Purification: silica gel (EtOAc/n-hexane, 10 : 1 + 1% Et_3N) and RP-MPLC (RP-18, 25–40 µm) using a linear gradient of acetonitrile/water (5–100% acetonitrile). The product eluted at 50% acetonitrile to yield 170.1 mg (63%). R_f: 0.40 (EtOAc/n-hexane, 5 : 1). ^{1}H-NMR (400 MHz, Acetone-d_6): δ 8.07 (1H, s, H-6), 7.53–7.51 (2H, m, Ar–H), 7.42–7.39 (4H, m, Ar–H), 7.36–7.32 (2H, m, Ar–H), 7.26–7.23 (1H, m, Ar–H), 6.92–6.90 (4H, m, Ar–H), 6.28 (1H, t, ^3J = 6.8 Hz, H-1′), 4.59–4.58 (1H, m, H-3′), 4.12–4.09 (3H, m, H-4′; CCCH_2O), 3.80 (6H, s, OCH_3), 3.56–3.47 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.42–3.31 (2H, m, H-5′), 2.42–2.39 (2H, m, H-2′), 1.86–1.81 (3H, m, triam-H-7,11,15), 1.72–1.60 (14H, m, triam-H-2,3,4,5,6,12,13,14,16,18), 1.39–1.29 (6H, m, triam-H-8,10,17). ^{13}C-NMR (101 MHz, Acetone-d_6): δ 161.9, 159.7, 150.3, 146.0, 143.9, 136.9, 136.6, 131.0, 130.0 128.8, 127.7, 114.1, 99.8, 89.9, 87.6, 86.4, 78.6, 72.4, 72.0, 71.4, 71.3, 71.0, 69.9, 64.7, 60.5, 59.3, 55.6, 49.7, 46.9, 46.0, 42.5, 41.8, 41.2, 38.7, 38.3, 36.3, 35.9, 35.3, 28.1. HRMS: *m/z*: calcd for $C_{57}H_{65}N_2O_{11}^-$ ([M – H]$^-$): 953.4594, found: 953.4596.

General procedure for nucleotides (1a–c)

The nucleosides **6** (1.0 equiv) and 1,8-bis(dimethylamino)-naphthalene (proton sponge, 1.5 equiv) were dried overnight in vacuum, dissolved in trimethyl phosphate, and cooled to 0 °C. Freshly distilled phosphoryl chloride (20 equiv) was added dropwise with stirring. The mixture was stirred at 0 °C for 6 h. A 0.5 M solution of bis(tri-n-butylammonium)pyrophosphate in anhydrous DMF (20 equiv) and tri-n-butylamine (40 equiv) were added simultaneously to the mixture. After 5 min, 1 M aqueous triethylammonium bicarbonate (TEAB buffer, pH 7.5) was added and the aqueous layer was washed with EtOAc (3 × 2 mL). The aqueous layer was concentrated in vacuo. and the resulting residue purified by ion-exchange chromatography [DEAE Sephadex™ A-25, linear gradient of TEAB buffer (0.1 M to 1 M, 1000 mL), flow 2 mL min^{-1}] and further purified by RP-MPLC (RP-18, 40–63 µm) using a gradient of 5% (200 mL), 20% (200 mL) and 40% (200 mL) acetonitrile in 50 mM aq. triethylammonium acetate (TEAA buffer, pH 7.0).

5-[12-(Adamantyl-1-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine-5′-triphosphate (1a)

The reaction was carried out with 5-[12-(Adamantyl-1-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine **6a** (45 mg, 82 µmol), 1,8-bis(dimethylamino)naphthalene (proton sponge, 43 mg, 0.20 mmol), phosphoryl chloride (200 µL), solution of bis(tri-n-butylammonium)pyrophosphate (3.5 mL, 1.7 mmol) and tri-n-butylamin (0.8 mL, 3.3 mmol) in 2.0 mL trimethylphosphate. Yield: 6.0 mg of triethylammonium salt (6% estimated by UV absorption). ^{1}H-NMR (600 MHz, D_2O): δ 8.19 (1H, s, H-6), 6.27 (1H, t, ^3J = 6.4 Hz, H-1′), 4.64–4.61 (1H, m, H-3′), 4.48 (2H, m, CCCH_2O), 4.25–4.15 (3H, m, H-4′, H-5′), 3.82–3.67 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.21–3.18 (24H, m, (HN(CH_2CH$_3$)$_3$)$_4^+$), 2.42–2.39 (2H, m, H-2′), 2.11 (m, 3H, adam-H-3,5,7), 1.75 (6H, m, adam-H-2,8,9), 1.69–1.60 (6H, m, adam-H-4,6,10), 1.30 (36H, t, ^3J = 6.8 Hz, (HN(CH$_2$CH_3)$_3$)$_4^+$). ^{31}P-NMR (162 MHz, D_2O): δ –10.6 (1P, m, P_γ), –11.7 (1P, m, P_α), –22.7 (1P, m, P_β). HRMS: *m/z*: calcd for $C_{28}H_{42}N_2O_{18}P_3^-$ ([M – H]$^-$): 787.1651, found: 787.1651.

5-[12-(Diamantyl-4-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine-5′-triphosphate (1b)

The reaction was carried out with 5-[12-(Diamantyl-4-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine **6b** (33 mg, 55 µmol), 1,8-bis(dimethylamino)naphthalene (proton sponge, 21.5 mg, 0.10 mmol), phosphoryl chloride (100 µL), solution of bis(tri-n-butylammonium)pyrophosphate (2.2 mL, 1.1 mmol) and tri-n-butylamin (0.5 mL, 2.2 mmol) in 2.0 mL trimethylphosphate. Yield: 3.5 mg of triethylammonium salt (5% estimated by UV absorption). ^{1}H-NMR (600 MHz, D_2O): δ = 8.19 (1H, s, H-6), 6,28 (1H, t, ^3J = 6.4 Hz, H-1′), 4.65–4.63 (1H, m, H-3′), 4.47 (2H, m, CCCH_2O), 4.22–4.09 (3H, m, H-4′, H-5′), 3.82–3.65 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.23–3.19 (24H, m, (HN(CH_2CH$_3$)$_3$)$_4^+$), 2.42–2.39 (2H, m, H-2′), 1.93 (3H, m, diam-H-2,6,12), 1.72–1.67 (m, 16H, diam-H-1,3,5,7,8,9,10,11,13,14), 1.28 (36H, t, ^3J = 6.8 Hz, (HN(CH$_2$CH_3)$_3$)$_4^+$). ^{31}P-NMR (162 MHz, D_2O): δ –10.8 (1P, m, P_γ), –11.5 (1P, m, P_α), –23.1 (1P, m, P_β). HRMS: *m/z*: calcd for $C_{32}H_{46}N_2O_{18}P_3^-$ ([M – H]$^-$): 839.1964, found: 839.1963.

5-[12-(Triamantyl-9-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine-5′-triphosphate (1c)

The reaction was carried out with 5-[12-(Triamantyl-9-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-2′-deoxyuridine **6c** (34 mg, 52 µmol), 1,8-bis(dimethylamino)naphthalene (proton sponge, 21.5 mg, 0.10 mmol), phosphoryl chloride (95 µL), solution of bis(tri-n-butylammonium)pyrophosphate (2.0 mL, 1.0 mmol) and tri-n-butylamin (0.5 mL, 2.1 mmol) in 2.0 mL trimethylphosphate. Yield: 3.2 mg of triethylammonium salt (5% estimated by UV absorption). ^{1}H-NMR (600 MHz, D_2O): δ 8.20 (1H, s, H-6), 6.26 (1H, t, ^{3}J = 6,4 Hz, H-1′), 4.65–4.62 (1H, m, H-3′), 4.44 (2H, m, CCCH_2O), 4.23–4.11 (3H, m, H-4′, H-5′), 3.79–3.61 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.26–3.22 (24H, m, (HN(CH_2CH_3)$_3$)$_4^+$), 2.43–2.39 (2H, m, H-2′), 1.87–1.85 (3H, m, triam-H-7,11,15), 1.72–1.60 (20H, m, triam-H-2,3,4,5,6,8, 10,12,13,14,16,17,18), 1.30 (36H, t, ^{3}J = 6.8 Hz, (HN(CH$_2$CH_3)$_3$)$_4^+$). ^{31}P-NMR (162 MHz, D_2O): δ –10.6 (1P, m, P_γ), –11.6 (1P, m, P_α), –22.9 (1P, m, P_β). HRMS: *m/z*: calcd for $C_{36}H_{50}N_2O_{18}P_3^-$ ([M – H]$^-$): 891.2277, found: 891.2277.

General procedure for Phosphoramidite (2a–c)

A solution of 5-[12-(diamondoidyloxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **8** (1.0 equiv) in anhydrous dichloromethane was stirred at 0 °C. N-ethyldiisopropylamine (3.0 equiv) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (2.0 equiv) were added dropwise with stirring. The mixture was stirred at 0 °C for 15 min. The reaction was allowed to stir at room temperature for 3 h and quenched with 5 mL anhydrous methanol. The residue was washed with ice-cold water (2 × 20 mL) and once with ice-cold brine. The organic layer was dried over magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was then purified by flash chromatography.

5-[12-(Adamantyl-1-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine-3′-(cyanoethyl-*N*,*N*′-diisopropyl)phosphoramidite (2a)

The reaction was carried out with 5-[12-(Adamantyl-1-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **8a** (85.1 mg, 0.10 mmol), N-ethyldiisopropylamine (51 µL, 0.30 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropyl-chlorophosphor-amidite (45 µL, 0.20 mmol) in 5 mL anhydrous dichloromethane. Purification: silica gel (EtOAc/n-hexane, 10 : 1 + 1% Et_3N). Yield: 74.0 mg (70%). R_f: 0.59 (EtOAc/n-hexane, 5 : 1). ^{1}H-NMR (400 MHz, Acetone-d_6): δ 8.07 (0.9H, s, H-6), 8.06 (0.1H, s, H-6), 7.53–7.51 (2H, m, Ar–H), 7.42–7.39 (4H, m, Ar–H), 7.36–7.32 (2H, m, Ar–H), 7.27–7.23 (1H, m, Ar–H), 6.92–6.90 (4H, m, Ar–H), 6.30–6.27 (1H, m, H-1′), 4.75–4.70 (1H, m, H-3′), 4.21–4.19 (1H, m, H-4′) 4.12–4.11 (2H, CCCH_2O), 3.80 (6H, s, OCH_3), 3.57–3.48 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.42–3.36 (2H, m, H-5′), 2.80–2.75 (4H, m, OCH_2CH_2CN), 2.63–2.50 (2H, m, H-2′), 2.09 (3H, m, adam-H-3,5,7), 1.72 (6H, m, adam-H-2,8,9), 1.66–1.58 (6H, m, adam-H-4,6,10), 1.26–1.10 (m, 14H, N(CH(CH_3)$_2$)$_2$). ^{13}C-NMR (101 MHz, Acetone-d_6): δ 161.8, 159.7, 150.2, 145.9, 143.9, 136.7, 136.5, 131.0, 128.9, 128.8, 127.7, 114.1, 99.9, 90.0, 87.6, 86.3, 78.6, 72.2, 72.0, 71.3, 71.2, 71.0, 69.8, 64.3, 60.1, 59.2, 55.6, 42.3, 40.8, 37.2, 31.4, 24.9, 24.8, 20.8, 20.7. ^{31}P-NMR (162 MHz, Acetone-d_6): δ 148.4, 148.2. ESI-MS: *m/z*: 1086.4 [M + Cl]$^-$.

5-[12-(Diamantyl-4-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine-3′-(cyanoethyl-*N*,*N*′-diisopropyl)phosphoramidite (2b)

The reaction was carried out with 5-[12-(Diamantyl-4-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **8b** (90.3 mg, 0.10 mmol), N-ethyldiisopropylamine (51 µL, 0.30 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropyl-chlorophosphor-amidite (45 µL, 0.20 mmol) in 5 mL anhydrous dichloromethane. Purification: silica gel (EtOAc/n-hexane, 7 : 1 + 1% Et_3N). Yield: 83.0 mg (75%). R_f: 0.62 (EtOAc/n-hexane, 5 : 1). ^{1}H-NMR (400 MHz, Acetone-d_6): δ 8.10 (0.5H, s, H-6), 8.08 (0.5H, s, H-6), 7.54–7.51 (2H, m, Ar–H), 7.43–7.39 (4H, m, Ar–H), 7.37–7.32 (2H, m, Ar–H), 7.27–7.24 (1H, m, Ar–H), 6.93–6.90 (4H, m, Ar–H), 6.31–6.26 (1H, m, H-1′), 4.77–4.71 (1H, m, H-3′), 4.26–4.20 (1H, m, H-4′) 4.15–4.07 (2H, CCCH_2O), 3.80 (6H, s, OCH_3), 3.57–3.45 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.44–3.36 (2H, m, H-5′), 2.80–2.63 (4H, m, OCH_2CH_2CN), 2.61–2.52 (2H, m, H-2′), 1.92 (3H, m, diam-H-2,6,12), 1.75–1.69 (16H, m, diam-H-1,3,5,7,8,9,10,11,13,14), 1.28–1.10 (14H, m, N(CH(CH_3)$_2$)$_2$). ^{13}C-NMR (101 MHz, Acetone-d_6): δ 161.8, 159.7, 150.2, 145.9, 143.9, 136.7, 136.5, 131.0, 128.9, 128.8, 127.7, 114.1, 99.9, 90.0, 87.6, 86.3, 78.5, 72.0, 71.5, 71.3, 71.2, 71.0, 69.8, 64.3, 60.5, 59.7, 59.5, 59.2, 55.6, 42.6, 40.7, 40.5, 38.0, 37.6, 26.6, 24.9, 24.8, 20.8, 20.7. ^{31}P-NMR (162 MHz, Acetone-d_6): δ 148.4, 148.2. ESI-MS: *m/z*: 1138.8 [M + Cl]$^-$.

5-[12-(Triamantyl-9-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine-3′-(cyanoethyl-*N*,*N*′-diisopropyl)phosphoramidite (2c)

The reaction was carried out with 5-[12-(Triamantyl-9-oxyl)-(4,7,10-trioxadodec-1-ynyl)]-5′-*O*-(4,4′-dimethoxytrityl)-2′deoxyuridine **8c** (95.5 mg, 0.10 mmol), N-ethyldiisopropylamine (51 µL, 0.30 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropyl-chlorophosphor-amidite (45 µL, 0.20 mmol) in 5 mL anhydrous dichloromethane. Purification: silica gel (EtOAc/n-hexane, 5 : 1 + 1% Et_3N). Yield: 92.0 mg (80%). R_f: 0.65 (EtOAc/n-hexane, 5 : 1). ^{1}H-NMR (400 MHz, Acetone-d_6): δ 8.10 (0.2H, s, H-6), 8.07 (0.8H, s, H-6), 7.53–7.51 (2H, m, Ar–H), 7.43–7.39 (4H, m, Ar–H), 7.36–7.32 (2H, m, Ar–H), 7.27–7.23 (1H, m, Ar–H), 6.93–6.90 (4H, m, Ar–H), 6.31–6,27 (1H, m, H-1′), 4.77–4.69 (1H, m, H-3′), 4.27–4.19 (1H, m, H-4′) 4.17–4.07 (CCCH_2O), 3.80 (6H, s, OCH_3), 3.57–3.46 (12H, m, OCH_2CH_2OCH_2CH_2OCH_2CH_2O), 3.44–3.36 (2H, m, H-5′), 2.80–2.75 (4H, m, OCH_2CH_2CN), 2.60–2.52 (2H, m, H-2′), 1.86–1.81 (3H, m, triam-H-7,11,15), 1.75–1.59 (14H, m, triam-H-2,3,4,5,6,12,13,14,16,18), 1.39–1.29 (6H, m, triam-H-8,10,17), 1.21–1.10 (14H, m, N(CH(CH_3)$_2$)$_2$). ^{13}C-NMR (101 MHz, Acetone-d_6): δ 161.8, 159.7, 150.2, 145.9, 143.9, 136.7, 136.5, 131.0, 128.9, 128.8, 127.7, 114.1, 99.9, 90.0, 87.6, 86.3, 78.5, 72.3, 72.0, 71.3, 71.2, 71.0, 69.8, 64.3, 60.4, 59.7, 59.5, 59.3, 55.6, 49.7, 46.8, 45.9, 42.5, 41.1, 40.8, 38.7, 38.3, 36.2, 35.9, 35.3, 28.1, 24.9, 24.8, 20.8, 20.7. ^{31}P-NMR (162 MHz, Acetone-d_6): δ 148.4, 148.2. ESI-MS: *m/z*: 1191.0 [M + Cl]$^-$.

Primer extension

A primer extension reaction (20 µL) contained: 1 × KlenTaq reaction buffer (50 mM Tris · HCl pH 9.2, 16 mM $(NH_4)_2SO_4$, 2.5 mM $mgCl_2$, 0.1% Tween 20), ^{32}P-labeled primer (150 nM), template (200 nM), dTDTP (100 µM each), dATP, dCTP, dGTP (100 µM each), DNA-polymerase (KlenTaq wild-type, 50 nM each). First primer and template were annealed in 1 × reaction buffer by heating the probes to 95 °C for 2 min and stepwise cooling to 0 °C. Afterwards the primer template complex, nucleotides and DNA polymerase were incubated at 60 °C for 30 min. The reactions were quenched by the addition of PAGE gel-loading buffer (45 µL, 80% formamide, 20 mm EDTA, 0.1% bromophenol blue, 0.1% xylene cyanole FF) and the product mixture was analysed by 12% denaturing polyacrylamide gel. Visualization was performed by phosphorimaging.

Primer: BRAF-23C (23mer)

5′-GAC CCA CTC CAT CGA GAT TTC TC-3′

Template: BRAF-35C (35mer)

3′-CTG GGT GAG GTA GCT CTA AAG AGG GCA CGG TCG GC-5′

Template: SO-69-NNNA (69mer)

3′-CTG GGT GAG GTA GCT CTA AAG AGA CTT ACG GAC TGA CGC ATT TAC TTA GCC ATT CAT TTA TCT AGG GAT-5′

Template: SO-69-NA (69mer)

3′-CTG GGT GAG GTA GCT CTA AAG AGA CAT ACA GAC AGA CAT ACA GAT ACA GAT ACA GAT ACA GAT ACA GAT-5′

Oligonucleotide synthesis

The oligonucleotides were prepared on a synthesizer (Applied Biosystems, 392 DNA/RNA Synthesizer) utilizing chemicals obtained from J.T. Baker and Applied Biosystems. The synthesis began with a dC bonded to the solid phase (polystyrene, Applied Biosystems, LV 200), in the trityl-off mode. After completion of synthesis, the solid phase was dried under vacuum. The oligonucleotide was cleaved from the solid support by treatment with ammonia (33%) at 55 °C overnight and the solvent was removed under vacuum. The residue was taken up in ultrapure water and separated by filtration from the solid phase and subsequently purified by preparative PAGE. The identity of the oligonucleotides was verified by ESI mass spectrometry.

Circular dichroism (CD)-measurements

CD spectra were measured on a Jasco J-715 instrument in a phosphate-buffer (20 mM K_2HPO_4/KH_2PO_4, pH = 7.0, 1.0 M NaCl) at 25 °C. Before the CD measurements, a heating step to 95 °C for 5 min followed by slowly cooling to 4 °C was performed to DNA duplex formation. For background subtraction a spectrum of the phosphate-buffer was separately measured. The scanning speed was 20 nm/min. The spectrum results from the sum of 10 measurements subtracting the spectrum of the phosphate-buffer.

Melting temperature (T_m)-measurements

Melting curves were recorded on Varian Cary 100 bio UV/Vis Spectrophotometer. 10 µM of the sample (same as for CD spectra) contained 1 × phosphate-buffer (20 mM K_2HPO_4/KH_2PO_4, pH = 7.0, 1.0 M NaCl). The duplex DNA samples were heated to 80 °C for 2 min and cooled with a gradient of 1.0 °C s^{-1} to the final temperature of 25 °C prior to the measurements. Data were taken from three individual cooling/heating cycles. The melting temperatures (T_m values in °C) were calculated using the first negative derivative of intensity over temperature.

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Notes and references

1 Review: S. Weisbrod and A. Marx, *Chem. Commun.*, 2008, 5675–5685.
2 G. Giller, T. Tasara, B. Angerer, K. Mühlegger, M. Amacker and H. Winter, *Nucleic Acids Res.*, 2003, **31**, 2630.
3 (*a*) O. Thum, S. Jäger and M. Famulok, *Angew. Chem., Int. Ed.*, 2001, **40**, 3990; (*b*) S. Obeid, A. Baccaro, W. Welte, K. Diederichs and A. Marx, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 21327.
4 Reviews: H. Schwertfeger, A. A. Fokin and P. R. Schreiner, *Angew. Chem.*, 2008, **120**, 1038; H. Schwertfeger, A. A. Fokin and P. R. Schreiner, *Angew. Chem., Int. Ed.*, 2008, **47**, 1022.
5 A. A. Fokin, B. A. Tkachenko, P. A. Gunchenko, D. V. Gusev and P. R. Schreiner, *Chem.–Eur. J.*, 2005, **11**, 7091.
6 (*a*) P. R. Schreiner, N. A. Fokina, B. A. Tkachenko, H. Hausmann, M. Serafin, J. E. P. Dahl, R. M. K. Carlson, S. G. Liu and A. A. Fokin, *J. Org. Chem.*, 2006, **71**, 6709; (*b*) P. R. Schreiner, A. A. Fokin, B. A. Tkachenko, E. Vass, M. Olmstead, D. Bläser, R. Boese, H. P. Reisenauer, J. E. P. Dahl and R. M. K. Carlson, *J. Am. Chem. Soc.*, 2009, **131**, 11292.
7 J. E. Dahl, S. G. Liu and R. M. K. Carlson, *Science*, 2002, **299**, 96 Admantane, diamantane, and triamantane are also available through chemical synthesis, see strategies and references in ref. 4.
8 (*a*) B. A. Tkachenko, N. A. Fokina, L. V. Chernish, J. E. P. Dahl, S. G. Liu, R. M. K. Carlson, A. A. Fokin and P. R. Schreiner, *Org. Lett.*, 2006, **8**, 1767; (*b*) A. A. Fokin, T. E. Shubina, P. A. Gunchenko, S. D. Isaev, A. G. Yurchenko and P. R. Schreiner, *J. Am. Chem. Soc.*, 2002, **124**, 10718; (*c*) P. R. Schreiner, A. A. Fokin, O. Lauenstein, Y. Okamoto, T. Wakita, C. Rinderspacher, G. H. Robinson, J. K. Vohs and C. F. Campana, *J. Am. Chem. Soc.*, 2002, **124**, 13348.
9 (*a*) W. K. Yang, J. D. Fabbri, T. M. Willey, J. R. I. Lee, J. E. Dahl, R. M. K. Carlson, P. R. Schreiner, A. A. Fokin, B. A. Tkachenko, N. A. Fokina, W. Meevasana, N. Mannella, K. Tanaka, X. J. Zhou, T. van Buuren, M. A. Kelly, Z. Hussain, N. A. Melosh and Z.-X. Shen, *Science*, 2007, **316**, 1460; (*b*) S. Roth, D. Leuenberger, J. Osterwalder, J. E. P. Dahl, R. M. K. Carlson, B. A. Tkachenko, A. A. Fokin, P. R. Schreiner and M. Hengsberger, *Chem. Phys. Lett.*, 2010, **495**, 102.
10 (*a*) L. Wanka, C. Cabrele, M. Vanejews and P. R. Schreiner, *Eur. J. Org. Chem.*, 2007, 1474; (*b*) A. A. Fokin, A. Merz, N. A. Fokina, H. Schwertfeger, S. G. Liu, J. E. P. Dahl, R. M. K. Carlson and P. R. Schreiner, *Synthesis*, 2009, 909.
11 A. Baccaro and A. Marx, *Chem.–Eur. J.*, 2010, **16**, 218.
12 P. Čapek, H. Cahová, R. Pohl, M. Hocek, C. Gloeckner and A. Marx, *Chem.–Eur. J.*, 2007, **13**, 6196.
13 S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum and M. Famulok, *J. Am. Chem. Soc.*, 2005, **127**, 15071.
14 T. Ohbayashi, M. Kuwahara, M. Hasegawa, T. Kasamatsu, T. Tamura and H. Sawai, *Org. Biomol. Chem.*, 2005, **3**, 2463.
15 S. H. Weisbrod and A. Marx, *Chem. Commun.*, 2007, 1828.
16 A. Baccaro, S. H. Weisbrod and A. Marx, *Synthesis*, 2007, **13**, 1949.
17 S. Obeid, M. Yulikov, G. Jeschke and A. Marx, *Angew. Chem.*, 2008, **120**, 6886; S. Obeid, M. Yulikov, G. Jeschke and A. Marx, *Angew. Chem., Int. Ed.*, 2008, **47**, 6782.
18 H. Sawai, A. N. Ozaki, F. Satoh, T. Ohbayashi, M. M. Masud and H. Ozaki, *Chem. Commun.*, 2001, 2604.
19 S. Ikonen, H. Macíčková-Cahová, R. Pohl, M. Šanda and M. Hocek, *Org. Biomol. Chem.*, 2010, **8**, 1194.

20 M. Hocek and M. Fojta, *Org. Biomol. Chem.*, 2008, **6**, 2233.
21 G. M. Dubowchik, L. Padilla, K. Edinger and R. A. Firestone, *J. Org. Chem.*, 1996, **61**, 4676.
22 T. Courtney, D. E. Johnston, M. A. McKervey and J. J. Rooney, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2691.
23 H. Duddeck, F. Hollowood, A. Karim and M. A. McKervey, *J. Chem. Soc., Perkin Trans. 2*, 1979, 360.
24 Z.-B. Li, Z.-H. Wu, K. Chen, F. T. Chin and X.-Y. Chen, *Bioconjugate Chem.*, 2007, **18**, 1987.
25 G. Lu, S. Lam and K. Burgess, *Chem. Commun.*, 2006, 1652.
26 M. J. Robins and P. J. Barr, *Tetrahedron Lett.*, 1981, **22**, 421.
27 K. Sonogashira, Y. Tohda and N. Hagihara, *Tetrahedron Lett.*, 1975, **16**, 4467.
28 J. D. Kahl and M. M. Greenberg, *J. Am. Chem. Soc.*, 1999, **121**, 597.
29 T. Kovács and L. Ötvös, *Tetrahedron Lett.*, 1988, **29**, 4525.
30 M. Yoshikava, T. Kato and T. Takenish, *Tetrahedron Lett.*, 1967, **8**, 5065.
31 J. Ludwig, *Biochim. Biophys. Acad. Sci. Hung.*, 1981, **16**, 131.
32 A. Rospigliosi, R. Ehlich, H. Hoerber, A. Middelberg and G. Moggridge, *Langmuir*, 2007, **23**, 8264.
33 R. H. E. Hudson and A. Ghorbani-Choghamarani, *Synlett*, 2007, 870.
34 N. Usman, K. K. Ogilvie, M.-Y. Jiang and R. J. Cedergrens, *J. Am. Chem. Soc.*, 1987, **109**, 7845.
35 N. D. Sinha, J. Biernat, J. McManus and H. Köster, *Nucleic Acids Res.*, 1984, **12**, 4539.
36 M. Chandra, S. Keller, Y. Luo and A. Marx, *Tetrahedron*, 2007, **63**, 8576.
37 C. Gloeckner, K. B. M. Sauter and A. Marx, *Angew. Chem.*, 2007, **119**, 3175; C. Gloeckner, K. B. M. Sauter and A. Marx, *Angew. Chem., Int. Ed.*, 2007, **46**, 3115.
38 M. J. Gait, *An introduction to modern methods of DNA synthesis. In Oligonucleotide Synthesis: A practical approach.*, IRL Press, Oxford, 1984.
39 F. Eckstein, *Oligonucleotide and analogues: A practical approach*, IRL Press, Oxford, 1991.
40 I. Detmer, D. Summerer and A Marx, *Chem. Commun.*, 2002, 2314.
41 S. L. Beaucage and M. H. Caruthers, *Tetrahedron Lett.*, 1981, **22**, 1859.
42 S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, **48**, 2223.
43 S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 6123.
44 L. J. McBride and M. H. Caruthers, *Tetrahedron Lett.*, 1983, **24**, 245.
45 M. H. Caruthers, *Science*, 1985, **230**, 281.
46 M. H. Caruthers, A. D. Barone, S. L. Beaucage, D. R. Dodds, E. F. Fisher, L. J. McBride, M. Matteucci, Z. Stabinsky and J.-Y. Tang, *Methods Enzymol.*, 1987, **154**, 287.
47 O. Dahl, J. Nielson and B. H. Dahl, *Nucleic Acids Res.*, 1987, **15**, 1729.

