# Synthesis of Thymidine Dimers from 5′-*O*-Aminothymidine

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**Abstract:** The synthesis of modified oligonucleotides is of great importance for various therapeutic and diagnostic applications. The facile secondary structure formation of *N*-oxyamide-linked peptide analogues and the high nucleophilicity of the aminooxy function prompted us to prepare *O*-amino nucleoside derived dinucleosides. Herein, the efficient synthesis of three novel thymidine dimers with *N*-oxyamide, oxime and oxyamine linkages via a convergent approach from a common 5′-*O*-aminothymidine is reported.

**Key words:** dinucleosides, *O*-amino nucleoside, thymidine, *N*-oxyamide, oxime, oxyamine

Modified oligonucleotides (ONs) are being investigated for various therapeutic and diagnostic applications because of their capability to cause selective inhibition of gene expression by binding to the target DNA/RNA sequences through mechanisms such as anti-gene, antisense, RNA interference, or to restore gene function by sterically blocking the access of cellular machinery to pre-mRNA and mRNA.[1,2] The availability of synthetic ONs has led to major advances in molecular biology; however, limited clinical success has been achieved with ONs, mainly due to their instability toward nucleases, lack of target specificity, and poor cellular uptake and targeted delivery. Therefore, the development of novel synthetic ONs with appropriate modifications and improved properties is of great importance. Nonionic analogues of nucleic acids are promising for the treatment of viral diseases and cancer since nonionic phosphate mimics could increase cellular permeability and resistance to extra- and intracellular nucleases. A number of phosphodiester replacements have been reported, including amide,[3] thioacetamide,[4] triazole,[5] amine,[6] formacetal,[7] thioformacetal,[7b,8] dimethylenesulfone,[9] *N*-acylsulfamide,[10] oxyamine or methylene(methylimino),[11] amino acid,[12] C=C double bond,[13] and silyl,[14] as well as oxyamide[15] analogues (Figure 1). These three- to six-atom-linked dinucleosides have been incorporated into standard oligonucleotides, with the ability to anneal to complementary DNA/RNA.

Recently, it has been demonstrated that O-aminopeptides can easily form intramolecular hydrogen bonds with turn and helice structures, and a new family of foldamers has been developed from α-, β- and γ-aminooxy acids.[16] Furthermore, the aminooxy group, thanks to its high nucleophilicity, reacts readily with aldehydes or carboxylic acids, resulting in the formation of a highly stable oxime or *N*-oxyamide linkage. Aminooxy-functionalized nucleosides[11,17] and oligonucleotides[18] have been reported in the development of methylene(methylimino)-linked oligonucleotide mimics, in prodrug design and in immobilization in the fabrication of microarrays. A *N*-oxyamide-linked thymidine dimer **M** (Figure 1) has been incorporated into a DNA oligomer which annealed to complementary DNA with nearly the same affinity as the natural sequence.[15] These results prompted us to prepare novel *O*-amino nucleoside derived dinucleosides. Latterly, we[19] and others[20] have developed glycoaminooxy acids as a new class of sugar building blocks, with inter-

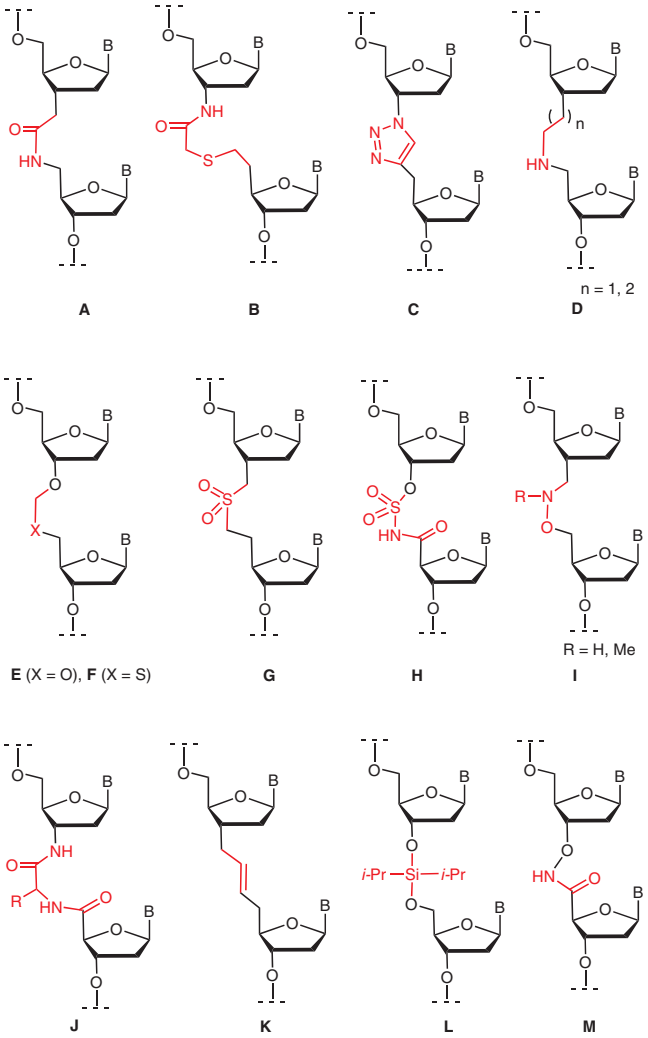


**Figure 1** Structures of nonionic dinucleoside analogues

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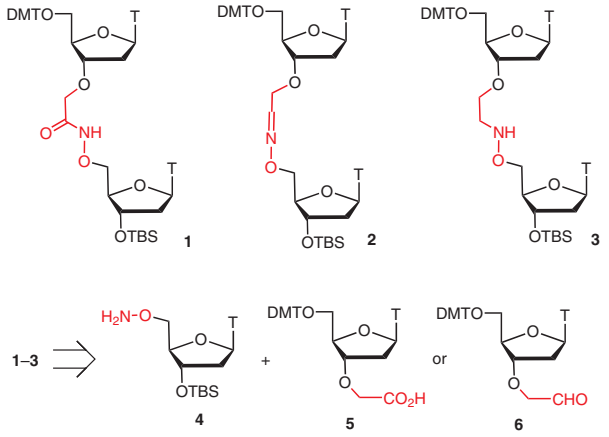
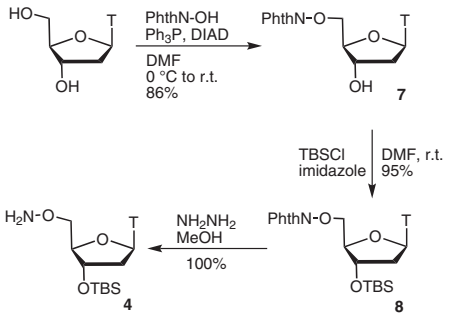
esting secondary structures for their oligomers.[20] Ribonucleoside aminooxy acids have also been prepared through N-glycosylation of glycoaminooxy acids.[21] As part of a continuing program on the synthesis of modified nucleosides and oligonucleotides, we decided to synthesize thymidine dimers **1**–**3** with *N*-oxyamide, oxime and oxyamine linkages (Scheme 1).



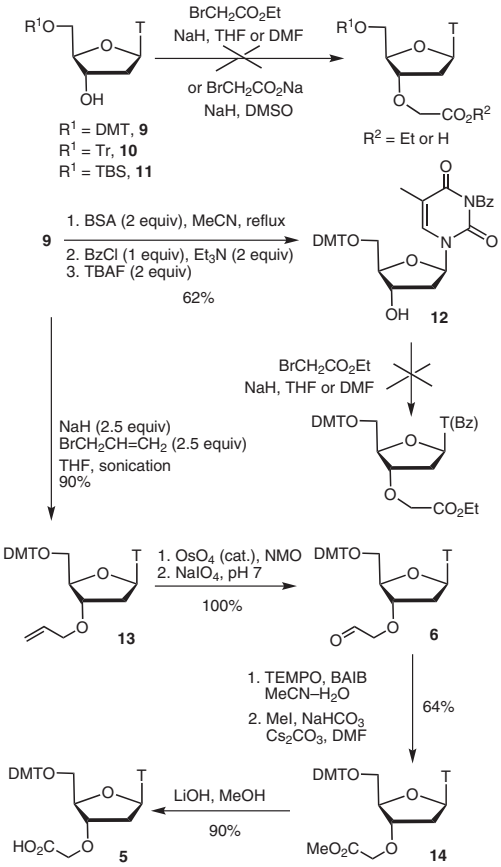

**Scheme 1** Structures and retrosynthesis of novel thymidine dinucleosides **1**–**3**

Thymidine dinucleosides **1**–**3** are accessible from 5′-*O*-aminothymidine **4** and thymidine carboxylic acid **5** or aldehyde **6**. We first prepared the common 5′-*O*-amino nucleoside **4**. To introduce the aminooxy function, commercial thymidine was reacted with *N*-hydroxyphthalimide using the Mitsunobu reaction.[11c] Slow addition of diisopropyl azodicarboxylate (1 drop every 10 seconds) allowed us to increase the yield of **7** to 86% (Scheme 2). The 3′-hydroxy group was then protected with the *tert*-butyldimethylsilyl (TBS) group. Hydrazinolysis led to the 5′-*O*-aminothymidine **4**.




**Scheme 2** Synthesis of 5′-*O*-aminothymidine **4**

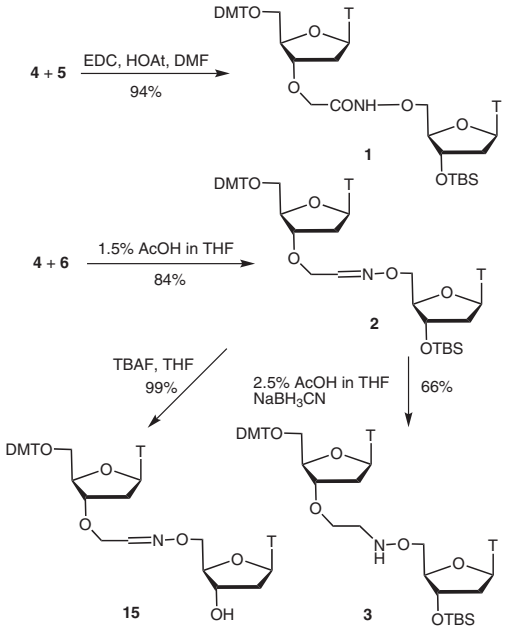
To synthesize the thymidine carboxylic acid **5**, we first envisaged an O-alkylation with ethyl bromoacetate (Scheme 3); however, this reaction failed to give the O-alkylated product with dimethoxytrityl-, trityl- or TBS-protected thymidines **9**–**11**, whatever the quantity of reagents, the nature of the solvent (THF or DMF) and the reaction time. N-Alkylation mainly occurred in all tested conditions, as proved by the $^{13}C$ NMR signal of N–$CH_2$ at 42.5 to 43.1




**Scheme 3** Synthesis of thymidine carboxylic acid and aldehyde derivatives **5** and **6**

ppm.[22] Treatment of thymidine **9** with sodium bromoacetate under Greenberg's conditions[23] also gave the N-alkylation product. We then decided to protect the N3 position of the thymidine with a benzoyl group, by temporary silylation of the 3′-hydroxy using *N*,*O*-bis(trimethylsilyl)acetamide (BSA), followed by benzoylation and desilylation. This one-pot procedure allowed us to rapidly obtain the protected intermediate **12**;[24] however, reaction of **12** with sodium hydride and ethyl bromoacetate appeared to be very slow and incomplete. As a consequence, the *O*-allylthymidine **13** was chosen as the intermediate for the preparation of both the carboxylic acid and aldehyde derivatives **5** and **6**. Thus, reaction of thymidine **9** with allyl bromide under ultrasonic conditions gave the desired product **13** in 90% yield.[25] Oxidative cleavage of the allyl group allowed the formation of the aldehyde **6** in quantitative yield. Radical oxidation (TEMPO, BAIB) led to the corresponding carboxylic acid derivative which was not pure enough to be engaged in further reactions. As purification was not easy because of the polarity of this compound and the acid sensitivity of the dimethoxytrityl (DMT) group, the obtained intermediate was converted into the pure methyl ester **14** in 64% yield (from aldehyde **6**). Addition of cesium carbonate (0.5 equiv) was expedient for completion of the esterification. The subsequent saponification gave the carboxylic acid derivative **5** in 90% yield.

Thymidine carboxylic acid **5** was then reacted with 5′-*O*-aminothymidine **4** with EDC/HOAt as coupling reagents to afford the *N*-oxyamide-linked dimer **1** in 94% yield (Scheme 4). It is to be noted that replacement of HOAt by HOBt led to the formation of several unidentified products. Formation of the oxime bond usually necessitated acidic conditions. Due to the presence of the very acid-sensitive DMT group in the aldehyde **6**, we firstly tried the condensation reaction in a mixture of acetate buffer (pH 5) and tetrahydrofuran (2:1); however, no reaction occurred because of poor solubility. Fortunately, the reaction worked smoothly in anhydrous *N*,*N*-dimethylformamide or tetrahydrofuran, in the presence of acetic acid. The best yield was obtained with 1.5% acetic acid in tetrahydrofuran, leading to the desired oxime **2** as a mixture of *E*/*Z*-isomers (3:2) in 84% yield. Desilylation of **2** gave the dimer **15** in 99% yield. Reduction of oxime **2** with sodium cyanoborohydride in the presence of 2.5% acetic acid in tetrahydrofuran led to the oxyamine-linked thymidine dimer **3** in 66% yield (Scheme 4).




**Scheme 4** Synthesis of thymidine dimers **1**–**3**

In summary, this paper describes the efficient synthesis of three novel thymidine dimers with *N*-oxyamide, oxime and oxyamine linkages via a convergent approach from the 5′-*O*-aminothymidine **4**. This methodology should be applicable to the synthesis of other *O*-amino nucleoside derived dinucleosides. These dinucleosides are ready to be incorporated into natural oligonucleotide sequences to study their annealing properties with complementary DNA or RNA.

All air-sensitive reactions were carried out under argon. Column chromatography was performed on E. Merck silica gel 60 (230–400 mesh). Analytical thin-layer chromatography was performed on E. Merck aluminum precoated plates of silica gel 60 F-254 with detection by UV and by spraying with 6 N $H_2SO_4$ and heating for ca. 2 min at 300 °C. NMR spectra were recorded in acetone-$d_6$ or $CD_3OD$ on a JEOL DX 400 spectrometer. Optical rotations were measured using a Jasco P-2000 polarimeter. High-resolution mass spectra (HRMS) were recorded on an MA1212 instrument using standard conditions (ESI, 70 eV).

**5′-*O*-Phthalimidothymidine (7)[11c]**

To a soln of thymidine (5.09 g, 21.0 mmol) in anhyd DMF (55 mL) under argon at 0 °C were added $Ph_3P$ (7.04 g, 26.9 mmol) and PhthNOH (4.46 g, 27.3 mmol), followed by the slow addition (1 drop/10 s) of a soln of DIAD (6.10 mL, 30.9 mmol) in anhyd DMF (10 mL). The mixture was stirred at r.t. for 15 h, then concentrated. The residue was dissolved in $CH_2Cl_2$ (50 mL) and poured into a mixture of $H_2O$–ice (250 mL); then, the resulting mixture was stirred for 30 min. Filtration of the formed precipitate afforded **7** as a white solid; yield: 7.01 g (86%); mp 222 °C.

$R_f$ = 0.50 ($CH_2Cl_2$–MeOH, 9:1).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.86 (d, $J$ = 0.9 Hz, 3 H, $CH_3^T$), 2.19 (ddd, $J$ = 5.5, 8.3, 14.2 Hz, 1 H, H-2′a), 2.25 (ddd, $J$ = 2.3, 6.0, 14.2 Hz, 1 H, H-2′b), 4.21–4.24 (m, 1 H, H-4′), 4.46 (dd, $J$ = 3.0, 10.1 Hz, 1 H, H-5′a), 4.50 (dd, $J$ = 4.6, 10.1 Hz, 1 H, H-5′b), 4.62–4.65 (m, 1 H, H-3′), 5.14 (d, $J$ = 4.1 Hz, 1 H, OH), 6.39 (dd, $J$ = 6.0, 8.3 Hz, 1 H, H-1′), 7.74 (d, $J$ = 0.9 Hz, 1 H, H-6), 7.90 (s, 4 H, Phth), 10.68 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.4 ($CH_3^T$), 40.3 (C-2′), 72.1 (C-3′), 78.6 (C-5′), 85.5 (C-1′, C-4′), 110.9 (C-5), 123.9 (Phth), 129.8 (Cq), 135.4 (Phth), 136.3 (C-6), 151.3, 163.8 (C-2, C-4, CO Phth).

**3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-phthalimidothymidine (8)**

To a suspension of **7** (498 mg, 1.29 mmol) in anhyd DMF (2.5 mL) under argon was added imidazole (229 mg, 3.37 mmol) followed by the dropwise addition of a soln of TBSCl (252 mg, 1.67 mmol) in anhyd DMF (2.5 mL). The mixture was stirred at r.t. for 15 h, then concentrated. Purification by flash column chromatography (EtOAc–petroleum ether, 1:1) afforded **8** as a white solid; yield: 610 mg (95%); mp 201 °C.

$[\alpha]_D^{22}$ +97.4 (*c* 0.80, acetone).

$R_f$ = 0.61 (EtOAc–petroleum ether, 7:3).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.19 (s, 6 H, 2 × $CH_3^{TBS}$), 0.94 (s, 9 H, *t*-$Bu^{TBS}$), 1.86 (d, $J$ = 0.9 Hz, 3 H, $CH_3^T$), 2.26 (ddd, $J$ = 2.7, 6.0, 13.7 Hz, 1 H, H-2′a), 2.32 (ddd, $J$ = 6.0, 8.3, 13.7 Hz, 1 H, H-2′b), 4.23–4.24 (m, 1 H, H-4′), 4.48 (dd, $J$ = 3.0, 10.3 Hz, 1 H, H-5′a), 4.53 (dd, $J$ = 3.9, 10.3 Hz, 1 H, H-5′b), 4.86–4.88 (m, 1 H, H-3′), 6.37 (dd, $J$ = 6.2, 8.1 Hz, 1 H, H-1′), 7.74 (d, $J$ = 0.9 Hz, 1 H, H-6), 7.90 (s, 4 H, Phth), 10.04 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –4.7 ($CH_3^{TBS}$), 14.4 ($CH_3^T$), 18.5 (Cq, *t*-$Bu^{TBS}$), 26.1 (*t*-$Bu^{TBS}$), 41.0 (C-2′), 73.6 (C-3′), 78.2 (C-5′), 85.7 (C-4′), 85.9 (C-1′), 111.1 (C-5), 124.0 (Phth), 130.0 (Cq), 135.6 (Phth), 136.4 (C-6), 151.2 (C-2), 163.8 (Cq), 164.2 (C-4), 170.9 (Cq).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{24}H_{31}N_3NaO_7Si$: 524.1829; found: 524.1823.

**5′-*O*-Amino-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (4)**

To a suspension of **8** (250 mg, 0.50 mmol) in MeOH (2 mL) was added $NH_2NH_2{\cdot}H_2O$ (75 μL, 1.55 mmol). The suspension became clear; this was followed by the formation of a precipitate after 2 h. $Et_2O$ (10 mL) was then added. The mixture was washed with sat. aq $NaHCO_3$ (10 mL). The aqueous layer was extracted with $Et_2O$ (3 × 20 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product **4** (white solid; 195 mg, 100%) was pure enough for further reactions.

Mp 98 °C; $[\alpha]_D^{22}$ –0.86 (*c* 0.90, MeOH).

$R_f$ = 0.39 (EtOAc–petroleum ether, 1:1).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.12 (s, 6 H, 2 × $CH_3^{TBS}$), 0.91 (s, 9 H, *t*-$Bu^{TBS}$), 1.80 (d, *J* = 1.3 Hz, 3 H, $CH_3^T$), 2.22–2.25 (m, 2 H, H-2′a, H-2′b), 4.07–4.10 (m, 1 H, H-4′), 4.18 (dd, *J* = 3.6, 12.0 Hz, 1 H, H-5′a), 4.23 (dd, *J* = 4.5, 12.0 Hz, 1 H, H-5′b), 4.60–4.62 (m, 1 H, H-3′), 6.28–6.31 (m, 1 H, H-1′), 7.49 (d, *J* = 1.3 Hz, 1 H, H-6), 9.94 (s, 2 H, $ONH_2$), 10.06 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –4.7 ($CH_3^{TBS}$), 12.6 ($CH_3^T$), 18.5 (Cq, *t*-$Bu^{TBS}$), 26.1 (*t*-$Bu^{TBS}$), 40.8 (C-2′), 73.6 (C-3′), 73.7 (C-5′), 85.5 (C-1′), 86.7 (C-4′), 110.7 (C-5), 136.3 (C-6), 151.2 (C-2), 164.2 (C-4).

### 5′-*O*-(Dimethoxytrityl)thymidine (9)[26]

To a soln of thymidine (4.96 g, 20.5 mmol) in anhyd pyridine (100 mL) under argon was added $Et_3N$ (5.8 mL, 41.3 mmol) and DMTCl (7.7 g, 22.7 mmol). The mixture was stirred at refux for 1.5 h, then concentrated. The residue was taken up in EtOAc (100 mL), washed with sat. aq $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography ($CH_2Cl_2$–MeOH–$Et_3N$, 98:1:1) afforded **9** as a white solid; yield: 9.15 g (82%); mp 89 °C.

$R_f$ = 0.31 ($CH_2Cl_2$–MeOH, 9:1).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.46 (d, *J* = 1.4 Hz, 3 H, $CH_3^T$), 2.30 (ddd, *J* = 2.8, 6.0, 13.8 Hz, 1 H, H-2′a), 2.38 (ddd, *J* = 6.1, 7.8, 13.8 Hz, 1 H, H-2′b), 3.37 (d, *J* = 3.6 Hz, 2 H, H-5′a, H-5′b), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.01–4.07 (m, 1 H, H-4′), 4.51 (d, *J* = 4.1 Hz, 1 H, OH), 4.58–4.62 (m, 1 H, H-3′), 6.38 (dd, *J* = 6.0, 7.8 Hz, 1 H, H-1′), 6.88–6.92 (m, 4 H, H-Ar), 7.23–7.38 (m, 7 H, H-Ar), 7.48–7.50 (m, 2 H, H-Ar), 7.63 (d, *J* = 1.4 Hz, 1 H, H-6), 10.01 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.1 ($CH_3^T$), 41.1 (C-2′), 55.5 ($OCH_3^{DMT}$), 64.8 (C-5′), 72.5 (C-3′), 79.2 (Cq), 85.1 (C-1′), 87.1 (C-4′), 110.9 (C-5), 113.9, 127.7, 128.7, 129.0 and 131.0 (5 × C-Ar), 136.4 (C-6), 145.9 (Cq), 151.2 (C-2), 159.7 (Cq), 164.2 (C-4).

### 3-*N*-Benzoyl-5′-*O*-(dimethoxytrityl)thymidine (12)[24]

To a soln of **9** (508 mg, 0.93 mmol) in anhyd MeCN (15 mL) under argon was added BSA (455 μL, 1.84 mmol). The mixture was stirred at reflux for 45 min, then cooled to r.t. $Et_3N$ (255 μL, 1.83 mmol) and BzCl (130 μL, 1.20 mmol) were added and the mixture was stirred for 15 h. TBAF (740 mg, 2.83 mmol) was introduced and the reaction mixture was stirred for 1 h, then concentrated. The residue was taken up in EtOAc (20 mL), washed with sat. aq $NaHCO_3$ (2 × 10 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (petroleum ether–EtOAc, 1:1) afforded **12** as a white solid; yield: 375 mg (62%); mp 92 °C.

$R_f$ = 0.61 (EtOAc–petroleum ether, 7:3).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.51 (d, *J* = 1.4 Hz, 3 H, $CH_3^T$), 2.38 (ddd, *J* = 3.3, 6.4, 13.5 Hz, 1 H, H-2′a), 2.48 (ddd, *J* = 6.2, 7.3, 13.5 Hz, 1 H, H-2′b), 3.41 (d, *J* = 3.2 Hz, 2 H, H-5′a, H-5′b), 3.80 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.07–4.10 (m, 1 H, H-4′), 4.53 (s, 1 H, OH), 4.63–4.65 (m, 1 H, H-3′), 6.34 (dd, *J* = 6.4, 7.3 Hz, 1 H, H-1′), 6.90–6.94 (m, 4 H, H-Ar), 7.25–7.29 (m, 1 H, H-Ar), 7.33–7.40 (m, 7 H, H-Ar), 7.50–7.60 (m, 4 H, H-Ar), 7.73–7.77 (m, 2 H, H-Ar), 7.82 (d, *J* = 1.4 Hz, 1 H, H-6).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.1 ($CH_3^T$), 41.3 (C-2′), 55.5 ($OCH_3^{DMT}$), 64.6 (C-5′), 72.3 (C-3′), 85.8 (C-1′), 87.4 (C-4′), 110.9 (C-5), 114.0, 127.8, 128.7, 129.0, 130.1, 131.0 and 131.1 (7 × C-Ar), 132.9 (Cq), 135.8 (C-6), 136.4 (C-Ar), 136.6 (Cq), 137.0 (Cq), 143.9 (Cq), 145.9 (Cq), 150.1 (C-2), 159.7 (Cq), 163.5 (C-4), 170.3 (Cq).

### 3′-*O*-Allyl-5′-*O*-(dimethoxytrityl)thymidine (13)[25c]

To a suspension of 60% NaH (182 mg, 4.55 mmol) in anhyd THF (10 mL) under argon at 0 °C was added **9** (988 mg, 1.82 mmol). After sonication for 20 min, allyl bromide (400 μL, 4.62 mmol) was added and the reaction mixture was stirred at r.t. for 3 h. The mixture was poured into sat. aq $NaHCO_3$ (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were then washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (EtOAc–petroleum ether, 1:1, + 1% $Et_3N$) afforded **13** as a white solid; yield: 954 mg (90%); mp 85 °C.

$[\alpha]_D^{22}$ +16.2 (*c* 0.90, acetone).

$R_f$ = 0.44 (EtOAc–petroleum ether, 7:3).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.47 (d, *J* = 1.4 Hz, 3 H, $CH_3^T$), 2.35 (ddd, *J* = 5.5, 7.8, 13.5 Hz, 1 H, H-2′a), 2.41 (ddd, *J* = 2.5, 6.0, 13.5 Hz, 1 H, H-2′b), 3.39 (d, *J* = 3.7 Hz, 2 H, $OCH_2$), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.02 (dd, *J* = 5.5, 13.3 Hz, 1 H, H-5′a), 4.07 (dd, *J* = 5.5, 13.3 Hz, 1 H, H-5′b), 4.12–4.14 (m, 1 H, H-4′), 4.37–4.39 (m, 1 H, H-3′), 5.14 (m, 1 H, $=CH_2^{allyl}$), 5.26 (m, 1 H, $=CH_2^{allyl}$), 5.85–5.98 (m, 1 H, $=CH^{allyl}$), 6.31 (dd, *J* = 6.0, 7.8 Hz, 1 H, H-1′), 6.89–6.93 (m, 4 H, H-Ar), 7.24–7.39 (m, 7 H, H-Ar), 7.47–7.50 (m, 2 H, H-Ar), 7.61 (d, *J* = 1.4 Hz, 1 H, H-6), 10.00 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.2 ($CH_3^T$), 38.2 (C-2′), 55.5 ($OCH_3^{DMT}$), 64.8 ($CH_2^{allyl}$), 70.6 (C-5′), 79.9 (C-3′), 84.6 (C-4′), 85.3 (C-1′), 87.5 (Cq), 111.0 (C-5), 114.0, 116.9, 127.7, 128.7, 129.0 and 131.0 (6 × C-Ar), 135.8 ($CH^{allyl}$), 136.5 (C-6), 145.9 (Cq), 151.2 (C-2), 159.7 (Cq), 164.2 (C-4).

### 5′-*O*-(Dimethoxytrityl)-3′-*O*-(formylmethyl)thymidine (6)[25c]

To a soln of **13** (1.63 g, 2.79 mmol) in a mixture of acetone–$H_2O$ (3:1, 12 mL) were added NMO (0.65 g, 5.58 mmol) and a soln of 4% $OsO_4$ in *t*-BuOH (900 μL). The mixture was stirred at r.t. for 2 h, then treated with $Na_2S_2O_5$ (0.98 g) and stirred for 30 min. $H_2O$ (50 mL) was then added. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The residue was dissolved in a mixture of acetone–phosphate buffer pH 7 (3:1, 22 mL), and $NaIO_4$ (1.50 g, 7.02 mmol) was added. The mixture was stirred at r.t. for 2 h, then filtered, and the precipitate was washed with EtOAc (50 mL). The separated aqueous layer was extracted with EtOAc (2 × 50 mL) and then the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The crude product **6** (beige solid; 1.64 g, 100%) was pure enough for further reactions.

Mp 123 °C; $[\alpha]_D^{22}$ +3.61 (*c* 0.80, acetone).

$R_f$ = 0.55 ($CH_2Cl_2$–MeOH, 9:1).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.45 (d, *J* = 0.9 Hz, 3 H, $CH_3^T$), 2.39 (ddd, *J* = 6.0, 8.5, 14.2 Hz, 1 H, H-2′a), 2.50 (ddd, *J* = 2.3, 6.0, 14.2 Hz, 1 H, H-2′b), 3.41–3.43 (m, 2 H, H-5′a, H-5′b), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.20–4.22 (m, 1 H, H-4′), 4.27 (s, 2 H, $OCH_2$), 4.46–4.48 (m, 1 H, H-3′), 6.33 (dd, *J* = 6.0, 8.5 Hz, 1 H, H-1′), 6.90–6.92 (m, 4 H, H-Ar), 7.25–7.37 (m, 7 H, H-Ar), 7.48–7.50 (m, 2 H, H-Ar), 7.60 (d, *J* = 0.9 Hz, 1 H, H-6), 9.64 (s, 1 H, CHO), 10.01 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.2 ($CH_3^T$), 38.0 (C-2′), 55.5 ($OCH_3^{DMT}$), 64.8 (C-5′), 75.4 ($OCH_2$), 81.7 (C-3′), 84.7 (C-4′), 87.5 (Cq), 87.6 (C-1′), 111.1 (C-5), 114.0, 127.8, 128.8, 129.0 and 131.0 (5 × C-Ar), 136.4 (C-6), 145.9 (Cq), 151.3 (C-2), 159.6 (Cq), 164.2 (C-4), 200.6 (CHO).

### 5′-*O*-(Dimethoxytrityl)-3′-*O*-(methoxycarbonylmethyl)thymidine (14)

To a soln of **6** (492 mg, 0.84 mmol) in a mixture of MeCN–$H_2O$ (1:1, 8 mL) were added BAIB (547 mg, 1.70 mmol) and TEMPO (27 mg, 0.17 mmol). The mixture was stirred at r.t. for 2 h, then diluted with EtOAc (15 mL) and washed with brine (15 mL). The aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was then engaged in ester formation.

To a soln of the acid derivative (500 mg, 0.84 mmol) in anhyd DMF (5 mL) under argon were added $NaHCO_3$ (222 mg, 2.64 mmol) and MeI (85 μL, 1.36 mmol). The mixture was stirred at r.t. for 15 h, then $Cs_2CO_3$ (143 mg, 0.43 mmol) was added to complete the reaction. The mixture was stirred for 24 h, then concentrated, and the residue was diluted with EtOAc (20 mL). The organic layer was washed with sat. aq $NaHCO_3$ (20 mL) and brine (20 mL), and then dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography ($CH_2Cl_2$–MeOH–$Et_3N$, 99:0:1, then 98:1:1) afforded **14** as an oil; yield: 345 mg (64%).

$[\alpha]_D^{22}$ +18.6 (*c* 1.6, acetone).

$R_f$ = 0.48 (EtOAc–petroleum ether, 7:3).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.46 (d, *J* = 0.9 Hz, 3 H, $CH_3^T$), 2.37 (ddd, *J* = 5.9, 8.3, 13.9 Hz, 1 H, H-2′a), 2.49 (ddd, *J* = 2.3, 6.0, 13.9 Hz, 1 H, H-2′b), 3.40 (dd, *J* = 3.7, 10.6 Hz, 1 H, H-5′a), 3.44 (dd, *J* = 3.7, 10.6 Hz, 1 H, H-5′b), 3.67 (s, 3 H, $COOCH_3$), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.17–4.20 (m, 1 H, H-4′), 4.22 (s, 2 H, $OCH_2$), 4.50–4.52 (m, 1 H, H-3′), 6.32 (dd, *J* = 6.0, 8.3 Hz, 1 H, H-1′), 6.89–6.93 (m, 4 H, H-Ar), 7.23–7.38 (m, 7 H, H-Ar), 7.47–7.50 (m, 2 H, H-Ar), 7.59 (d, *J* = 0.9 Hz, 1 H, H-6), 10.03 (s, 1 H, NH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.1 ($CH_3^T$), 38.0 (C-2′), 51.9 ($COOCH_3$), 55.5 ($OCH_3^{DMT}$), 64.8 (C-5′), 66.9 ($OCH_2$), 81.5 (C-3′), 84.7 (C-4′), 85.1 (C-1′), 87.5 (Cq), 111.0 (C-5), 114.0, 127.7, 128.7, 129.0 and 131.0 (5 × C-Ar), 136.3 (C-6), 136.6 (Cq), 145.9 (Cq), 151.2 (C-2), 159.7 (Cq), 164.1 (C-4), 171.2 (*C*OOCH$_3$).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{34}H_{36}N_2NaO_9$: 639.2319; found: 639.2303.

### 3′-*O*-(Carboxymethyl)-5′-*O*-(dimethoxytrityl)thymidine (5)[23]

To a soln of **14** (200 mg, 0.32 mmol) in MeOH (2 mL) was added LiOH (12 mg, 0.50 mmol). The reaction mixture was stirred at r.t. for 36 h, then quenched with sat. aq $NH_4Cl$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product **5** (oil; 176 mg, 90%) was pure enough for further reactions.

$[\alpha]_D^{22}$ +11.9 (*c* 1.50, MeOH).

$R_f$ = 0.13 ($CH_2Cl_2$–MeOH, 9:1).

$^1$H NMR (400 MHz, $CD_3OD$): δ = 1.36 (d, *J* = 1.4 Hz, 3 H, $CH_3^T$), 2.27–2.34 (m, 1 H, H-2′a), 2.50–2.54 (m, 1 H, H-2′b), 3.37–3.43 (m, 2 H, H-5′a, H-5′b), 3.77 (s, 6 H, 2 × $OCH_3^{DMT}$), 3.93 (s, 2 H, $OCH_2$), 4.21–4.25 (m, 1 H, H-4′), 4.38–4.41 (m, 1 H, H-3′), 6.31–6.34 (m, 1 H, H-1′), 6.85–6.87 (m, 4 H, H-Ar), 7.28–7.31 (m, 7 H, H-Ar), 7.41–7.43 (m, 2 H, H-Ar), 7.68 (d, *J* = 1.4 Hz, 1 H, H-6).

### 3′-*O*-(*N*-Carbonylmethyl)-5′-*O*-(dimethoxytrityl)thymidylyl-(3′→5′)-5′-*O*-amino-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (1)

To a soln of **5** (51 mg, 0.085 mmol) in anhyd DMF (1 mL) at 0 °C under argon were added HOAt (15 mg, 0.11 mmol) and EDC (11 mg, 0.10 mmol). The mixture was stirred for 10 min, then **4** (32 mg, 0.085 mmol) was added. The reaction was complete after stirring for 1 night at r.t. The mixture was diluted with EtOAc (10 mL) and washed with sat. aq $NaHCO_3$ (10 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography ($CH_2Cl_2$–MeOH–$Et_3N$, 99:0:1, then 98:1:1) afforded **1** as a white solid; yield: 76 mg (94%); mp 137 °C.

$[\alpha]_D^{22}$ +19.8 (*c* 1.10, acetone).

$R_f$ = 0.36 ($CH_2Cl_2$–MeOH, 9.5:0.5).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.13 (s, 6 H, 2 × $CH_3^{TBS}$), 0.92 (s, 9 H, *t*-Bu$^{TBS}$), 1.45 (d, *J* = 1.4 Hz, 3 H, $CH_3^T{}_A$), 1.84 (d, *J* = 0.9 Hz, 3 H, $CH_3^T{}_B$), 2.14–2.26 (m, 2 H, H-2′a$_B$, H-2′b$_B$), 2.34–2.42 (m, 2 H, H-2′a$_A$, H-2′b$_A$), 3.39 (dd, *J* = 3.4, 10.7 Hz, 1 H, H-5′a$_A$), 3.45 (dd, *J* = 3.4, 10.3 Hz, 1 H, H-5′b$_A$), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.05–4.07 (m, 1 H, H-4′$_B$), 4.10–4.15 (m, 4 H, H-5′a$_B$, H-5′b$_B$, $OCH_2$), 4.22–4.24 (m, 1 H, H-4′$_A$), 4.48–4.49 (m, 1 H, H-3′$_A$), 4.71–4.73 (m, 1 H, H-3′$_B$), 5.58 (s, 1 H, CO–NH–O), 6.31–6.36 (m, 2 H, H-1′$_A$, H-1′$_B$), 6.89–6.93 (m, 4 H, H-Ar), 7.23–7.27 (m, 1 H, H-Ar), 7.32–7.38 (m, 6 H, H-Ar), 7.47–7.51 (m, 2 H, H-Ar), 7.61 (d, *J* = 1.4 Hz, 1 H, H-6$_A$), 7.85 (d, *J* = 0.9 Hz, 1 H, H-6$_B$), 10.0 (s, 2 H, NH$_A$, NH$_B$).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –4.6 ($CH_3^{TBS}$), 12.2 ($CH_3^T{}_A$), 12.4 ($CH_3^T{}_B$), 18.5 (Cq, *t*-Bu$^{TBS}$), 26.1 ($CH_3$, *t*-Bu$^{TBS}$), 37.8 (C-2′$_A$), 41.2 (C-2′$_B$), 55.5 ($OCH_3^{DMT}$), 64.9 (C-5′$_A$), 68.8 ($OCH_2$), 73.8 (C-3′$_B$), 76.6 (C-5′$_B$), 82.2 (C-3′$_A$), 84.4 (C-4′$_A$), 85.1, 85.7 (C-1′$_A$, C-1′$_B$), 86.2 (C-4′$_B$), 87.6 (Cq), 111.1 (C-5$_A$, C-5$_B$), 114.0, 127.7, 128.7, 129.0 and 131.0 (5 × C-Ar), 136.3, 136.9 (C-6$_A$, C-6$_B$), 145.8 (Cq), 151.2 (C-2$_A$, C-2$_B$), 159.7 (Cq), 164.1, 164.3 (C-4$_A$, C-4$_B$), 166.5 (Cq).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{49}H_{61}N_5NaO_{13}Si$: 978.3927; found: 978.3894.

### Oxime Dinucleoside 2

To a soln of **6** (162 mg, 0.28 mmol) and **4** (105 mg, 0.28 mmol) in anhyd THF (10 mL) under argon was added AcOH (150 μL). The mixture was stirred at r.t. for 20 h, then neutralized with sat. aq $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography ($CH_2Cl_2$–MeOH–$Et_3N$, 98:1:1) afforded **2** as a white solid; yield: 220 mg (84%); mp 135 °C.

$R_f$ = 0.42 ($CH_2Cl_2$–MeOH, 9.5:0.5).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.11 (s, 6 H, 2 × $CH_3^{TBS}$), 0.91 (s, 9 H, *t*-Bu$^{TBS}$), 1.29 (d, *J* = 1.4 Hz, 3 H, $CH_3^T{}_A$), 1.79 (d, *J* = 0.9 Hz, 3 H, $CH_3^T{}_B$), 2.19–2.33 (m, 2 H, H-2′a$_B$, H-2′b$_B$), 2.35–2.50 (m, 2 H, H-2′a$_A$, H-2′b$_A$), 3.37–3.44 (m, 2 H, H-5′a$_A$, H-5′b$_A$), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.04–4.08 (m, 1 H, H-4′$_B$), 4.13–4.16 (m, 1 H, H-4′$_A$), 4.17–4.20 [t, *J* = 5.0 Hz, 1.2 H, OC*H*$_2$CH=N–O (*E*)], 4.24–4.31 (m, 2 H, H-5′a$_B$, H-5′b$_B$), 4.38–4.41 [dd, *J* = 3.4, 8.0 Hz, 0.8 H, OC*H*$_2$CH=N–O (*Z*)], 4.43–4.46 (m, 1 H, H-3′$_A$), 4.54–4.58 (m, 1 H, H-3′$_B$), 6.25–6.32 (m, 2 H, H-1′$_A$, H-1′$_B$), 6.89–6.95 [m, 4.4 H, H-Ar, CH=N–O (*Z*)], 7.23–7.27 (m, 1 H, H-Ar), 7.31–7.36 (m, 6 H, H-Ar), 7.47–7.51 (m, 3 H, H-Ar, H-6$_B$), 7.59–7.61 [m, 1.6 H, H-6$_A$, CH=N–O (*E*)], 10.0 (s, 2 H, NH$_A$, NH$_B$).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –4.7 ($CH_3^{TBS}$), 12.2 ($CH_3^T{}_A$), 12.7 ($CH_3^T{}_B$), 18.5 (Cq, *t*-Bu$^{TBS}$), 26.2 ($CH_3$, *t*-Bu$^{TBS}$), 37.9 (C-2′$_A$), 40.6 (C-2′$_B$), 55.5 ($OCH_3^{DMT}$), 64.3 [O*C*H$_2$CH=N–O (*Z*)], 64.8 (C-5′$_A$), 66.7 [O*C*H$_2$CH=N–O (*E*)], 73.4 (C-3′$_B$), 74.5 (C-5′$_B$), 75.0 (Cq), 81.0 (C-3′$_A$), 84.5 (C-4′$_A$), 85.2, 85.5 (C-1′$_A$, C-1′$_B$), 86.1 (C-4′$_B$), 87.5 (Cq), 110.0, 110.8 (C-5$_A$, C-5$_B$), 114.0, 127.7, 128.7, 129.0 and 131.0 (5 × C-Ar), 136.2, 136.4 (C-6$_A$, C-6$_B$), 145.8 (Cq), 148.6 (Cq), 151.2 (C-2$_A$, C-2$_B$), 159.7 (Cq), 164.1 (C-4$_A$, C-4$_B$).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{49}H_{62}N_5O_{12}Si$: 940.4164; found: 940.4151; *m*/*z* $[M + Na]^+$ calcd for $C_{49}H_{61}N_5NaO_{12}Si$: 962.3984; found: 962.3972.

### 5′-*O*-(Dimethoxytrityl)-3′-*O*-(*N*-ethyl)thymidylyl-(3′→5′)-5′-*O*-amino-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (3)

To a soln of dinucleoside **2** (52 mg, 0.055 mmol) in anhyd THF (1 mL) under argon were added AcOH (25 μL) and $NaBH_3CN$ (10 mg, 0.17 mmol). The mixture was stirred at r.t. for 24 h, then $NaBH_3CN$ (10 mg, 0.17 mmol) was added again. The mixture was stirred for 48 h, then diluted with EtOAc (10 mL) and washed with sat. aq $NaHCO_3$ (10 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography ($CH_2Cl_2$–MeOH–$Et_3N$, 98:1:1) afforded **3** as a white solid; yield: 34 mg (66%); mp 106 °C.

$[\alpha]_D^{22}$ +18.8 (*c* 0.7, acetone).

$R_f$ = 0.45 ($CH_2Cl_2$–MeOH, 9.5:0.5).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.11 (s, 6 H, 2 × $CH_3^{TBS}$), 0.91 (s, 9 H, *t*-$Bu^{TBS}$), 1.46 (d, *J* = 0.9 Hz, 3 H, $CH_3^T{}_A$), 1.80 (d, *J* = 0.9 Hz, 3 H, $CH_3^T{}_B$), 2.15–2.28 (m, 2 H, H-2′$a_B$, H-2′$b_B$), 2.32–2.46 (m, 2 H, H-2′$a_A$, H-2′$b_A$), 3.09–3.13 (m, 2 H, $OCH_2CH_2$NH–O), 3.36–3.43 (m, 2 H, H-5′$a_A$, H-5′$b_A$), 3.62–3.71 (m, 2 H, $OCH_2CH_2$NH–O), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 3.81–3.92 (m, 2 H, H-5′$a_B$, H-5′$b_B$), 3.99–4.02 (m, 1 H, H-4′$_B$), 4.13–4.15 (m, 1 H, H-4′$_A$), 4.33–4.35 (m, 1 H, H-3′$_A$), 4.50–4.53 (m, 1 H, H-3′$_B$), 6.26–6.31 (m, 2 H, H-1′$_A$, H-1′$_B$), 6.43–6.46 (t, 1 H, $CH_2$N*H*–O), 6.89–6.93 (m, 4 H, H-Ar), 7.23–7.27 (m, 1 H, H-Ar), 7.32–7.38 (m, 6 H, H-Ar), 7.48–7.50 (m, 2 H, H-Ar), 7.56 (d, *J* = 0.9 Hz, 1 H, H-6), 7.60 (d, *J* = 0.9 Hz, 1 H, H-6), 9.94 (s, 1 H, $NH_A$), 9.97 (s, 1 H, $NH_B$).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –4.6 ($CH_3^{TBS}$), 12.2 ($CH_3^T{}_A$), 12.6 ($CH_3^T{}_B$), 18.5 (Cq, *t*-$Bu^{TBS}$), 26.1 ($CH_3$, *t*-$Bu^{TBS}$), 38.0 (C-2′), 40.9 (C-2′), 52.2 ($OCH_2CH_2$NH–O), 55.5 ($OCH_3^{DMT}$), 64.9 (C-5′$_A$), 66.7 ($OCH_2CH_2$NH–O), 73.7 (C-3′$_B$), 74.4 (C-5′$_B$), 81.0 (C-3′$_A$), 84.5 (C-4′$_A$), 85.2, 85.5 (C-1′$_A$, C-1′$_B$), 86.4 (C-4′$_B$), 87.5 (Cq), 110.8, 111.0 (C-5$_A$, C-5$_B$), 114.0, 127.7, 128.7, 129.0 and 131.0 (5 × C-Ar), 136.3, 136.5 (C-6$_A$, C-6$_B$), 145.8 (Cq), 151.2 (C-2$_A$, C-2$_B$), 159.7 (Cq), 164.2 (C-4$_A$, C-4$_B$).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{49}H_{63}N_5NaO_{12}Si$: 964.4135; found: 964.4087.

**Oxime Dinucleoside 15**

To a soln of **2** (301 mg, 0.32 mmol) in THF (3 mL) was added TBAF (150 mg, 0.57 mmol). The mixture was stirred at r.t. for 1 night, then concentrated, diluted with EtOAc (30 mL) and washed with sat. aq NaCl (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography ($CH_2Cl_2$–MeOH–$Et_3N$, 98:1:1, then 97:2:1) afforded **15** as a white solid; yield: 261 mg (99%); mp 122 °C.

$R_f$ = 0.33 ($CH_2Cl_2$–MeOH, 9.5:0.5).

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 1.47 (d, *J* = 1.4 Hz, 3 H, $CH_3^T{}_A$), 1.81 (d, *J* = 1.4 Hz, 3 H, $CH_3^T{}_B$), 2.15–2.29 (m, 2 H, H-2′$a_B$, H-2′$b_B$), 2.36–2.48 (m, 2 H, H-2′$a_A$, H-2′$b_A$), 3.39–3.40 (m, 2 H, H-5′$a_A$, H-5′$b_A$), 3.79 (s, 6 H, 2 × $OCH_3^{DMT}$), 4.07–4.11 (m, 1 H, H-4′$_B$), 4.14–4.15 (m, 1 H, H-4′$_A$), 4.17–4.19 [t, *J* = 5.0 Hz, 1.2 H, $OCH_2$CH=N–O (*E*)], 4.25–4.30 (m, 2 H, H-5′$a_B$, H-5′$b_B$), 4.37–4.41 [m, 0.8 H, $OCH_2$CH=N–O (*Z*)], 4.44–4.48 (m, 2 H, H-3′$_A$, H-3′$_B$), 4.58–4.59 (m, 1 H, OH), 6.28–6.34 (m, 2 H, H-1′$_A$, H-1′$_B$), 6.89–6.93 [m, 4.4 H, H-Ar, CH=N–O (*Z*)], 7.23–7.28 (m, 1 H, H-Ar), 7.31–7.38 (m, 6 H, H-Ar), 7.44–7.50 (m, 3 H, H-Ar, H-6$_B$), 7.57–7.58 [m, 0.6 H, CH=N–O (*E*)], 7.61 (d, *J* = 1.4 Hz, 1 H, H-6$_A$), 10.00 (s, 2 H, $NH_A$, $NH_B$).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = 12.2 ($CH_3^T{}_A$), 12.7 ($CH_3^T{}_B$), 38.0 (C-2′$_A$), 40.4 (C-2′$_B$), 55.5 ($OCH_3^{DMT}$), 64.7 [$OCH_2$CH=N–O (*Z*)], 64.8 (C-5′$_A$), 66.6 [$OCH_2$CH=N–O (*E*)], 72.3 (C-3′$_B$), 75.0 (C-5′$_B$), 75.1 (Cq), 81.0 (C-3′$_A$), 84.5 (C-4′$_A$), 85.1, 85.2 (C-1′$_A$, C-1′$_B$), 86.1 (C-4′$_B$), 110.8, 111.1 (C-5$_A$, C-5$_B$), 114.0, 127.7, 128.7, 129.0 and 131.0 (5 × C-Ar), 136.3, 136.4 (C-6$_A$, C-6$_B$), 145.8 (Cq), 148.6 (Cq), 151.2 (C-2$_A$, C-2$_B$), 159.7 (Cq), 164.1 (C-4$_A$, C-4$_B$).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{43}H_{47}N_5NaO_{12}$: 848.3113; found: 848.3058.

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