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Synthesis of triazole-linked morpholino oligonucleotides *via* Cu^I catalysed cycloaddition†

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Triazole-linked morpholino (TLMO) oligonucleic acids were synthesised using the Cu^I catalysed (3 + 2) azide–alkyne cycloaddition (CuAAC) reaction. The modified DNA analogues were incorporated into 13-mer sequences *via* solid phase synthesis. UV melting experiments showed that the TLMO modification gives higher *T_m* values than the corresponding TLDNA modification.

Introduction

Click chemistry has recently emerged as a powerful tool in the field of nucleic acid research.¹ In particular, the Cu^I catalysed (3 + 2) azide–alkyne cycloaddition (CuAAC)² has been used to construct modified internucleotide linkages,³ to prepare nucleic acid conjugates,⁴ and as a strand ligation tool.⁵ Zerrouki *et al.*, designed a novel triazole-linked DNA analogue (TLDNA) **1** using the CuAAC for oligomer elongation,⁶ and this preliminary work has been significantly extended by Brown *et al.* The artificial TLDNA retains good aqueous solubility, is stable towards enzymatic degradation,^{3a} and can be read by polymerases,⁷ thus making it capable of *in vitro* transcription⁸ and rolling circle amplification.⁷ Furthermore, and perhaps most impressively, Brown has demonstrated that genes containing TLDNA **1** are functional *in vivo* in *Escherichia coli* and in human cells.⁹ Given the biocompatibility of the TLDNA **1** with DNA processing enzymes, it is curious that the thermal stability of complementary duplexes is reduced. A recent study on the structural basis of this phenomenon reported that the TLDNA modification leads to less optimal stacking interactions and distortion in the backbone at, and adjacent to, the site of the triazole.¹⁰ Whilst high melting temperatures are not required for all uses of modified nucleic acids, the formation of stable duplexes is a requirement for therapeutic applications of oligonucleotides, and as such TLDNAs **1** do not represent good drug candidates.

As part of our own research aimed at developing therapeutic nucleic acids, we wondered if the thermal stability of triazole-containing duplexes could be improved by the addition of further modifications to the backbone. Thus we decided to examine triazole-linked morpholino (TLMO) hybrid structures **2** (Fig. 1) as they could combine the ease of synthesis of the TLDNAs **1** with the increased melting temperatures associated with morpholino drug candidates.^{11,12} The TLMO hybrid **2** can be disconnected to reveal the azide **4** and the alkyne-substituted morpholine **3** as potential precursors for the proposed CuAAC reaction (Fig. 1).

Our initial route to **3** proceeded *via* the morpholine **6**, which was readily prepared from 5-methyl uridine **5** in good yield by oxidative cleavage and subsequent reductive amination¹³ (Scheme 1). Although the *N*-alkylation of **6** did produce

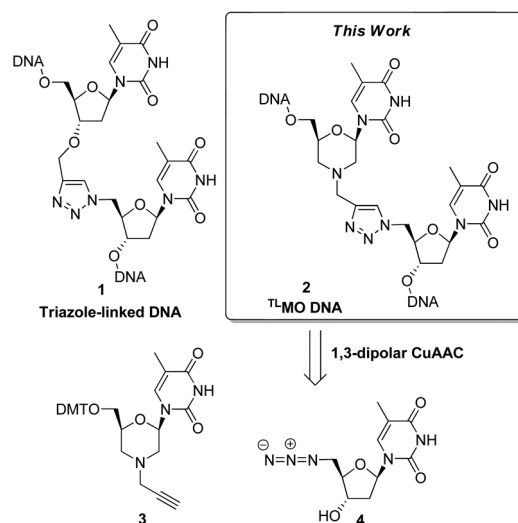


Fig. 1 Approach to triazole-linked morpholino (TLMO) hybrid structures **2**.

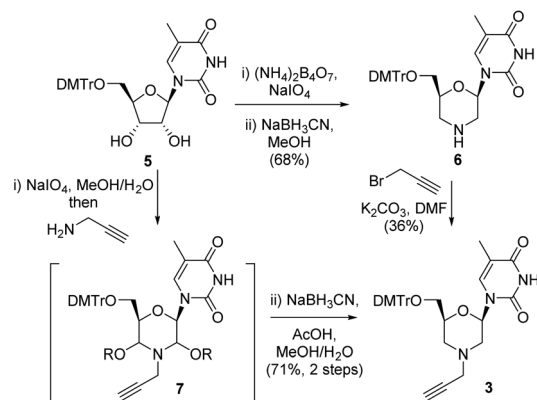
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† Electronic supplementary information (ESI) available: Full experimental details and copies of ¹H, ¹³C, ³¹P NMR spectra for all compounds. See DOI: 10.1039/c6ob00007j

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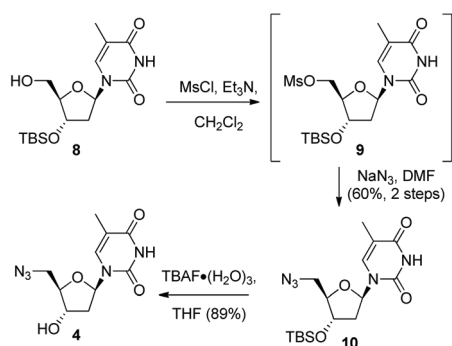


Scheme 1 Synthesis of the alkyne morpholine nucleoside **3**.

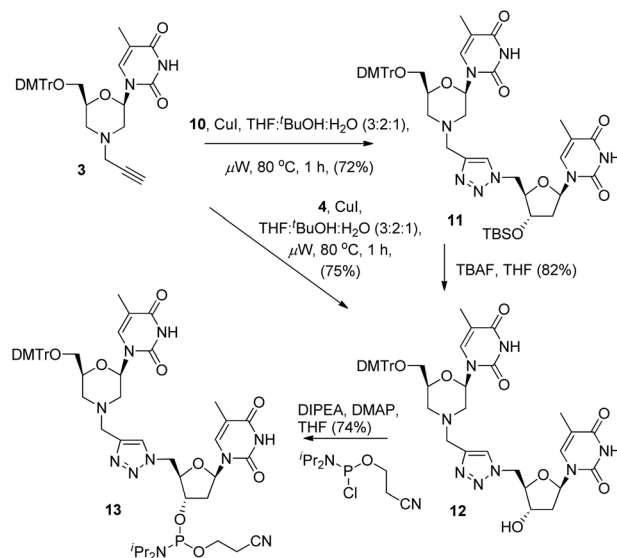
the desired *N*-propargyl morpholine **3**, only a low yield (36%) of the desired alkyne was obtained. The main side reaction was over alkylation of the thymine base in addition to *N*-alkylation of the morpholine, and an alternative route was explored. Thus, oxidative cleavage of **5** in the presence of propargylamine first gave **7**, which upon treatment with sodium cyanoborohydride/AcOH gave the desired product **3** in good overall yield (71%) (Scheme 1).

Following formation of the alkyne-containing morpholine nucleoside **3**, our attention then turned to the synthesis of the azide cycloaddition partner. Pleasingly, the 3'-TBSO-protected-5'-deoxy-5'-azido thymidine **10** was readily prepared from the alcohol **8** *via* a two-step sequence involving mesylate formation and displacement with sodium azide. The 3'-OH-5'-deoxy-5'-azido thymidine **4** was then synthesised from **10** *via* TBAF deprotection (Scheme 2). Alternatively, **4** could be accessed directly from thymidine *via* selective 5'-tosylation and subsequent azide displacement using the known procedure (38%, 2 steps).¹⁴

With the required coupling partners in hand, we next examined the key Cu-catalysed cycloaddition step. As we needed access the 3'-alcohol **12** to prepare phosphoramidite **13**, we explored two routes for its synthesis. The first was cycloaddition of **3** and the protected azide **10** (*i.e.* **3** + **10** → **11**), followed by TBS deprotection (**11** → **12**), and the second was initial deprotection (**10** → **4**, Scheme 2) followed by cyclo-



Scheme 2 Synthesis of 5'-deoxy-5'-azido thymidine **4**.



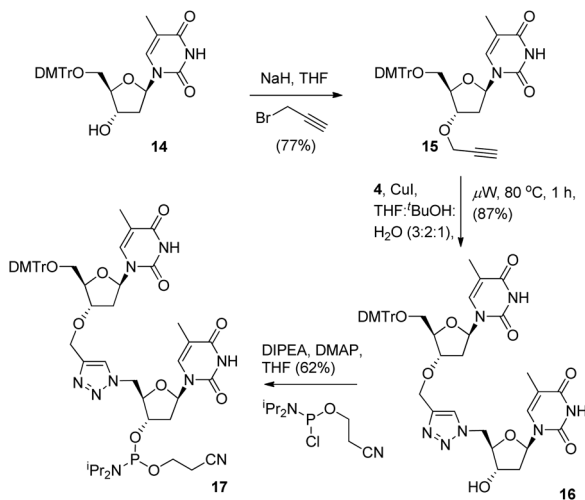
Scheme 3 Synthesis of 3'-cyanoethyl phosphoramidite **13**.

addition with **3**. A range of catalysts and solvents were initially screened, and it was quickly found that the use of copper(I) iodide in THF : *t*-BuOH : H₂O (3 : 2 : 1) with microwave heating (80 °C) was optimal (Scheme 3).^{3a} Under these conditions, cycloaddition of the acetylene **3** with the TBS-protected azide **10** gave the triazole-linked morpholino (^{TL}MO) dimer **11** in good yield, and TBAF deprotection of **11** gave the desired alcohol **12** in good yield (Scheme 3). We were pleased to find that the alternative cycloaddition of **4** with **3** also proceeded in good yield to give the alcohol **12** directly, and this was adopted as our favoured route due to an improved overall yield and easier of purification of **12** by column chromatography. Finally, the ^{TL}MO **12** was converted to the 3'-cyanoethyl phosphoramidite **13** (74%) under standard conditions (Scheme 3).

To provide a direct comparison of the new ^{TL}MO hybrid **2** to the triazole-linked DNA analogue (^{TL}DNA) **1**, we next prepared the phosphoramidite **17**. This reagent facilitates incorporation of the triazole modification **1** into oligonucleotide sequences *via* solid-phase synthesis as opposed to the fragment ligation method used previously by Brown *et al.*¹⁰ The phosphoramidite **17** was readily prepared from **14** *via* 3'-*O*-alkylation to give the alkyne **15**, Cu-catalysed cycloaddition with **4** to provide the triazole-containing dimer **16** and then conversion to **17** in the usual manner (Scheme 4).

Pleasingly, the modified phosphoramidites **13** and **17** were fully compatible with solid-phase oligonucleotide synthesis and we prepared the ^{TL}MO-containing oligomer **21** and the known ^{TL}DNA oligomer **22**¹⁰ in good yield (Table 1). Stock aqueous solutions (pH 7) of the oligomers **21** and **22** were readily prepared, and no adverse solubility issues were observed. As Brown *et al.* have already reported UV-melting data of **22** duplexed with its complementary DNA strand **18**,¹⁰ we also prepared **18** so that we could directly compare the *T*_m values of **18** + **21**, **18** + **22** and the unmodified duplex (**18** + **20**) under the same conditions. In order to assess the potential





Scheme 4 Synthesis of 3'-cyanoethyl phosphoramidite 17.

Table 1 Sequences of oligonucleotides synthesised

Identifier	Sequence 5' → 3'	m/z	
		Calculated	Found
18 (DNA)	d(GCTGCAAACGTCG)	3953.55	3953.73
19 (RNA)	GCUGCAAACGUCG	4133.49	4133.63
20	d(CGACGTTTGAGC)	3944.53	3944.72
21 ^a	d(CGACG ^T T ^T TGCAGC)	3944.64	3944.80
22 ^b	d(CGACG ^T ^ ^T TGCAGC)	3945.63	3945.78

^a **T^T** indicates the position of the morpholine-triazole modification 13. ^b **T[^]T** indicates the position of the triazole modification 17.

use of the ^{TL}MO-modification in therapeutically useful oligomers, we also synthesised the complimentary RNA oligonucleotide 19, as this simulates an intracellular mRNA target. The integrity of the oligomers 18–22 was confirmed by ESI mass spectrometry (Table 1) and HPLC (see ESI†).

Thermal stabilities of the ^{TL}MO 21, ^{TL}DNA 22, and unmodified DNA 20 duplexed with complimentary DNA 18 (Fig. 2) and RNA 19 (Fig. 3) were then determined by UV melting experiments¹⁵ (Table 2). Pleasingly, the *T_m* values of the control DNA 18:DNA 20 (62.4 °C) (entry 1, Table 2), and the DNA 18:^{TL}DNA 22 (55.1 °C) (entry 3, Table 2) duplexes were in close agreement with those reported previously by Brown (62.89 °C and 55.30 °C respectively).¹⁰ The ^{TL}MO-containing oligomer 21 duplexed to DNA 18 gave a *T_m* value of 56.1 °C (entry 2, Table 2), which represents a small increase (ΔT_m 1.0 °C) over that determined for 22, but still represents a significant decrease from the unmodified DNA (ΔT_m -6.3 °C). As mentioned above, duplexes with RNA provide a more meaningful comparison for future therapeutic applications and the *T_m* value of RNA 19 duplexed with unmodified DNA 20 was determined (58.5 °C) as a control (entry 4, Table 2). In contrast to

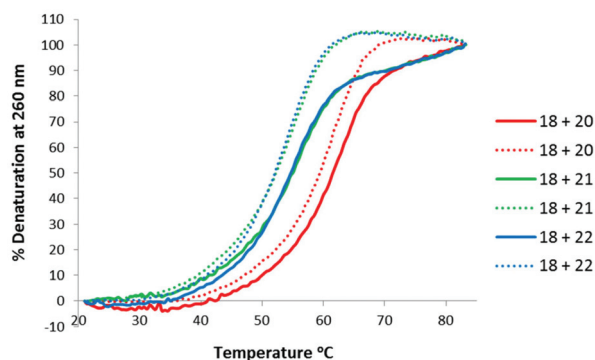


Fig. 2 UV-melting curves for oligomers (3 μM) 20, 21 and 22 duplexed with DNA 18. Dotted lines represent cooling curves.

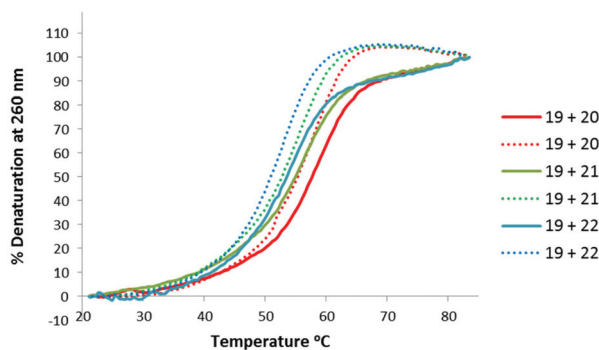


Fig. 3 UV-melting curves for oligomers (3 μM) 20, 21 and 22 duplexed with RNA 19. Dotted lines represent cooling curves.

Table 2 Thermal melting (*T_m*) data for oligonucleotide duplexes

Entry	Oligomers	<i>T_m</i> ^a °C	<i>T_m</i> ^b
1	18 + 20	62.4 (61.9)	N/A
2	18 + 21	56.1 (54.7)	-6.3 (-7.2)
3	18 + 22	55.1 (54.1)	-7.3 (-7.8)
4	19 + 20	58.5 (58.2)	N/A
5	19 + 21	56.6 (55.3)	-1.9 (-2.9)
6	19 + 22	54.1 (52.8)	-4.4 (-5.4)

^a *T_m* values for 3 μM oligo samples. Values in parentheses refer to cooling curves. ^b ΔT_m per modification relative to the control DNA 20.

the duplexes with DNA, the *T_m* of ^{TL}MO 21 (56.6 °C) was much closer to that of the unmodified DNA:RNA than was ^{TL}DNA 22 (54.1 °C) with RNA 19 (ΔT_m -1.9 °C for 21 vs. -4.4 °C for 22) (entries 5 and 6, Table 2), thus demonstrating that the addition of the morpholine modification can regain half of the *T_m* lost by incorporating the triazole internucleotide linkage.

Further structural studies are underway in order to fully assess the duplexes formed by ^{TL}MO-modified oligomers, before selecting the best candidates for biological evaluation.



Experimental[†]

5'-O-DMTr-morpholino thymidine (6)

5'-O-DMTr-5-methyluridine 5 (700 mg, 1.25 mmol) was dissolved in dry MeOH (10 mL) under an argon atmosphere. Ammonium baborate (328 mg, 2.50 mmol), sodium periodate (535 mg, 2.50 mmol) were added to the reaction mixture. After stirring for 3 h at room temperature, the mixture was filtered and sodium cyanoborohydride (155 mg, 2.50 mmol) was added to the filtrate in one portion with stirring. Stirring continued for 6 h followed by evaporation to afford a residue, which was dissolved in EtOAc (10 mL), washed with brine (3 × 10 mL). The organic phase was dried over MgSO₄, filtered and evaporated and purified by column chromatography eluting with (CHCl₃:MeOH, 25:1) to afford **6** as a colourless foam (460 mg, 68% over three steps); *R*_f 0.28 (CHCl₃:MeOH, 25:1); [α]_D²⁵ +47 (*c* 0.61, CHCl₃); ν_{max}/cm⁻¹ (CHCl₃) 3389, 2933, 2838, 2103, 1684, 1609, 1487 and 1455; ¹H NMR (400 MHz, CDCl₃) 7.47–7.42 (2H, m, Ar), 7.36–7.27 (6H, m, Ar), 7.32 (1H, s, C6H), 7.24–7.19 (1H, m, Ar), 6.84 (4H, d, *J* 8.9, Ar), 5.77 (1H, dd, *J* 10.0, 2.7, C1'H), 4.01 (1H, dtd, *J* 10.7, 4.9, 2.2, C4'H), 3.79 (6H, s, OCH₃), 3.27 (1H, dd, *J* 9.7, 5.1, C5'HH), 3.15 (1H, dd, *J* 12.5, 2.7, C2'HH), 3.11–3.02 (2H, m, C5'HH and C3'HH), 2.68–2.58 (2H, m, C3'HH, C2'HH), 1.95 (3H, s, CH₃); ¹³C NMR (100 MHz, CDCl₃) 164.1 (C), 158.5 (C), 150.4 (C), 144.8 (C), 135.9 (C), 135.8 (C), 135.4 (CH), 130.1 (CH), 130.0 (CH), 128.1 (CH), 127.8 (CH), 126.9 (CH), 113.1 (CH), 110.9 (C), 86.1 (C), 80.5 (CH), 78.0 (CH), 64.5 (CH₂), 55.2 (CH₃), 49.0 (CH₂), 46.9 (CH₂), 12.9 (CH₃); HRMS *m/z* (ES+) Found 566.2245 (M + Na, C₃₁H₃₃N₃NaO₆ requires 566.2245).

N'-propargyl-5'-O-DMTr-morpholino thymidine (3)

To a stirred solution of 5'-O-DMTr 5-methyluridine (**5**) (725 mg, 1.29 mmol) in MeOH (12 mL) under an argon atmosphere, was added a solution of sodium periodate (304 mg, 1.42 mmol) in water (2 mL) dropwise over 5 min, followed by propargyl amine (103 μL, 1.62 mmol) in one portion. The resulting solution was stirred at room temperature for 3 hours, during which time a white precipitate formed, the mixture was filtered. To the stirred solution of the filtrate was added sodium cyanoborohydride (162 mg, 2.58 mmol) followed by the dropwise addition of acetic acid (110 μL, 1.93 mmol). The reaction was stirred for 12 h at room temperature. The volatile organic were removed by evaporation. The residue was partitioned between sat. NaHCO₃ (50 mL) and EtOAc (50 mL), the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over MgSO₄, and evaporated. The residue was purified by silica gel chromatography, eluting with DCM:MeOH (40:1) to afford the title compound (529 mg, 71%) as a colourless foam; *R*_f 0.17 (DCM:MeOH 25:1); [α]_D²⁵ +30 (*c* 0.93, CHCl₃); ν_{max}/cm⁻¹ (CHCl₃) 3390, 3196, 2955, 1933, 1838, 1694, 1633, 1609, 1583, 1491, 1456; ¹H NMR (400 MHz, chloroform-d) δ 9.95

(1H, br s, NH), 7.48–7.44 (2H, m, Ar), 7.37–7.27 (6H, m, Ar), 7.32 (1H, s, C6H), 7.24–7.20 (1H, m, Ar), 6.84 (4H, d, *J* 8.9, Ar), 5.93 (1H, dd, *J* 9.8, 2.7, C1'H), 4.12 (1H, m, C4'H), 3.79 (6H, s, OCH₃), 3.45–3.44 (2H, m, NCH₂C≡CH), 3.34 (1H, dd, *J* 9.6, 5.3, C5'HH), 3.11 (1H, dd, *J* 9.6, 5.4, C5'HH), 2.97 (1H, br d, *J* 10.5, C3'H_AH_B), 2.84 (1H, br d, *J* 11.4, C2'HH), 2.32 (1H, t, *J* 2.3, C≡CH), 2.34–2.23 (2H, m, C3'HH, C2'HH), 1.96 (3H, s, CH₃); ¹³C NMR (101 MHz, chloroform-d) 164.1 (C), 158.6 (C), 150.3 (C), 144.8 (C), 136.0 (C), 135.8 (C), 135.6 (CH), 130.13 (CH), 130.09 (CH), 128.2 (CH), 127.9 (CH), 126.9 (CH), 113.2 (CH), 110.9 (C), 86.2 (C), 79.6 (CH), 77.4 (C), 75.6 (CH), 74.5 (CH), 64.6 (CH₂), 55.3 (CH₃), 54.6 (CH₂), 52.8 (CH₂), 46.4 (CH₂), 12.7 (CH₃); ¹H NMR (400 MHz, Benzene-d₆) δ 9.88 (1H, s, NH), 7.68 – 7.59 (2H, m, Ph), 7.52–7.40 (4H, m, Ar), 7.23–7.15 (2H, m, Ph), 7.11 – 7.01 (1H, m, Ph), 6.94 (1H, q, *J* 1.2, C6H), 6.81–6.71 (4H, m, Ar), 5.95 (1H, dd, *J* 9.7, 2.7, C1'H), 3.96–3.90 (1H, m, C4'H), 3.38 (1H, dd, *J* 9.6, 5.4, C5'HH), 3.30 (3H, s, OCH₃), 3.29 (3H, s, OCH₃) 3.17 (1H, dd, *J* 9.6, 5.1, C5'HH), 3.01 (1H, dd, *J* 17.5, 2.4, NCHHC≡CH), 2.92 (1H, dd, *J* 17.5, 2.4, NCHHC≡CH), 2.63 (1H, d, *J* 10.4, C2'HH), 2.47 (1H, d, *J* 10.9, C3'HH), 2.16 (1H, t, *J* 11.0, C3'HH), 2.16 (1H, t, *J* 10.4, C3'HH), 1.90 (1H, t, *J* 2.4, C≡CH), 1.66 (3H, d, *J* 1.2, CH₃); ¹³C NMR (101 MHz, Benzene-d₆) δ 163.9 (C4), 159.3 (2 × ArC), 150.3 (C2), 145.7 (PhC), 136.4 (ArC), 136.3 (ArC), 135.1 (C6H), 130.6 (4 × ArCH), 128.7 (2 × PhCH), 127.9 (2 × PhCH), 127.2 (PhCH), 113.6 (4 × ArCH), 110.8 (C5H), 86.7 (Ar₃CO), 80.2 (C1'H), 78.0 (C), 75.9 (C4'H), 74.3 (CH), 65.1 (C5'H₂), 54.8 (2 × OCH₃ and C2'H₂), 52.6 (C3'H₂), 46.3 (NCH₂C), 12.6 (CH₃). HRMS (ESI) C₃₄H₃₆N₃O₆ (M + H⁺) requires 582.2599, found 582.2569.

5'-O-Mesyl-3'-O-tert-butyl dimethylsilyl deoxythymidine (9)

To a stirred solution of 5'-OH-3'-O-tert-butyl dimethylsilyl deoxythymidine (1.06 g, 3.0 mmol) in dichloromethane (15 mL) at 0 °C was added triethylamine (0.84 mL, 6.0 mmol) followed by the dropwise addition of mesyl chloride (277 μL, 3.6 mmol). The resulting solution was stirred at 0 °C for 1 h, then warmed to room temperature, and stirred for a further 3 hours. The reaction was quenched by the addition of water (50 mL), the layers were separated, and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with sat NH₄Cl (50 mL), sat. NaHCO₃ (50 mL), brine (50 mL), dried over MgSO₄, and evaporated to afford the title compound (1.29 g, Quant.) as a yellow foam, which was used without further purification; ν_{max}/cm⁻¹ (CHCl₃) 3393, 3006, 2955, 2930, 2885, 1690, 1471, 1362, 1320, 1257, 1176, 1132, 1085 and 1062; ¹H NMR (400 MHz, chloroform-d) δ 9.20 (1H, s, NH), 7.31 (1H, q, *J* 1.2, C6H), 6.28 (1H, t, *J* 6.7, C1'H), 4.450 (1H, dd, *J* 11.2, 3.0, C5'HH) 4.45–4.38 (1H, m, C4'H), 4.36 (1H, dd, *J* 11.2, 3.6, C5'HH), 4.05 (1H, app. q, *J* 3.6, C3'H), 3.06 (3H, s, SO₂CH₃), 2.28 (1H, ddd, *J* 13.6, 6.4, 3.9, C2'HH), 2.17 (1H, dt, *J* 13.6, 6.8, C2'HH), 1.93 (3H, d, *J* 1.3, CH₃), 0.88 (9H, s, SiC(CH₃)₃), 0.09 (6H, s, Si(CH₃)₂); ¹³C NMR (101 MHz, chloroform-d) δ 163.9 (C4), 150.4 (C2), 135.6 (C6H), 111.7 (C5), 85.4 (C4'H), 84.3 (C1'H), 71.5 (C3'H), 68.5 (C6'H₂), 40.6 (C2'H₂), 37.8 (SO₂CH₃), 25.7 (SiC(CH₃)₃) 17.9, (SiC(CH₃)₃) 12.6 (CH₃), –4.6

[†]For general experimental details please see the ESI.[†]



(SiCH₃), -4.8 (SiCH₃); HRMS (ESI) C₁₇H₃₁N₂O₇SSi (M + H) requires 435.1616; found 436.1624 and C₁₇H₃₀N₂NaO₇SSi (M + Na) requires 457.1435; found 457.1444.

5'-Azide-3'-O-tert-butyl dimethylsilyl deoxythymidine (10)

A solution of 5'-O-mesyl-3'-O-tert-butyl dimethylsilyl deoxythymidine (9) (1.29 g, 3.0 mmol) and sodium azide (580 mg, 9.0 mmol) in dry DMF (12 mL) under argon was heated to 100 °C for 14 h. The reaction was cooled to room temperature, diluted with water (100 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine (3 × 75 mL), over MgSO₄, and evaporated. The residue was purified by silica gel chromatography, eluting with petrol: diethyl ether (1:1 to 0:1) to afford the title compound (686 mg, 61%) as a white foam; R_f 0.18 (petrol: diethyl ether 1:1); [α]_D²² +80 (c 0.78, CHCl₃); ν_{max}/cm⁻¹ (CHCl₃) 3087, 3062, 3010, 2928, 2855, 2104, 1959, 1701, 1670, 1624, 1554, 1509, 1480, 1448, 1381, 1349, 1309, 1249, 1178, 1153; ¹H NMR (500 MHz, chloroform-d) δ 8.95 (1H, s, NH), 7.31 (1H, q, J 1.2, C6H), 6.24 (1H, t, J 6.6, C1'H), 4.34 (1H, dt, J 7.0, 4.3, C3'H), 3.93 (1H, dt, J 4.4, 3.5, C4'H), 3.70 (1H, dd, J 13.3, 3.4, C5'HH), 3.49 (1H, dd, J 13.3, 3.6, C5'HH), 2.28 (1H, ddd, J 13.6, 6.6, 4.3, C2'HH), 2.16 (1H, dt, J 13.7, 6.9, C2'HH), 1.94 (3H, d, J 1.3, CH₃), 0.88 (9H, s, SiC(CH₃)₃), 0.08 (6H, s, Si(CH₃)₂); ¹³C NMR (126 MHz, chloroform-d) δ 163.8 (C4), 150.4 (C2), 135.5 (C6H), 111.5 (C5), 84.9 (C4'H, and C1'H), 71.8 (C3'H), 51.9 (C6'H₂), 40.8 (C2'H₂), 25.8 (SiC(CH₃)₃), 18.04 (SiC(CH₃)₃), 12.8 (CH₃), -4.54 (SiCH₃), -4.77 (SiCH₃); HRMS (ESI) C₁₆H₂₈N₅O₄Si (M + H) requires 382.1905; found 382.1910 and C₁₆H₂₇N₅NaO₄Si (M + Na) requires 404.1725; found 404.1737.

5'-Azide-3'-OH deoxythymidine (4)

To a stirred solution of 5'-azido-3'-O-tert-butyl dimethylsilyl deoxythymidine (10) (151 mg, 400 μmol) in THF (2.0 mL) under an argon atmosphere, was added tetrabutylammonium fluoride trihydrate (189 mg, 600 μmol). The resulting solution was stirred at room temperature for 12 hours, and then the volatile organics were evaporated. The residue was purified by silica gel chromatography, eluting with EtOAc: MeOH (19:1) to afford the title compound (95 mg, 89%) as a white foam; R_f 0.20 (EtOAc); [α]_D²² +114 (c 1.0, CHCl₃); ν_{max}/cm⁻¹ (CHCl₃); 3390, 3009, 2956, 2105, 1690, 1471, 1438, 1262; ¹H NMR (500 MHz, Methanol-d₄) δ 7.54 (1H, q, J 1.2, C6H), 6.26 (1H, t, J 6.8, C1'H), 4.34 (1H, dt, J 6.5, 4.1, C3'H), 3.96 (1H, dt, J 5.0, 3.8, C5'H), 3.63 (1H, dd, J 13.2, 3.7, C5'HH), 3.57 (1H, dd, J 13.2, 5.1, C5'HH), 2.31 (1H, dd, J 13.7, 6.6, C2'HH), 2.25 (1H, ddd, J 13.7, 6.6, 3.9, C2'HH), 1.89 (3H, d, J 1.3, CH₃). ¹³C NMR (126 MHz, Methanol-d₄) δ 166.3 (C4), 152.3 (C2), 137.7 (C6H), 111.9 (C5), 86.4 (C4'H or C1'H), 86.3 (C4'H or C1'H), 72.5 (C3'H), 53.4 (C6'H₂), 40.2 (C2'H₂), 12.5 (CH₃); HRMS (ESI) C₁₀H₁₄N₅O₄ (M + H) requires 268.1040; found 268.1044 and C₁₀H₁₄N₅NaO₄ (M + Na) requires 290.0859; found 290.0858.

¹¹L-Morpholino-3'-O-tert-butyl silyl dimer T-T (11)

To a microwave vial containing the morpholino thymidine (3) (290 mg, 500 μmol) and the azide thymidine (10) (190 mg,

500 μmol) in THF: ^tBuOH: H₂O (3:2:1 ratio, total volume 2 mL) was added copper iodide (47.0 mg, 250 μmol). The vial was sealed, stirred and irradiated in a Biotage microwave at 80 °C (approximately power of irradiation 16 W) for 3½ h. After cooling to room temperature the vial was removed, and the solvents were removed *in vacuo* to afford a residue, which was purified by silica gel chromatography, eluting with DCM: MeOH (40:1 to 30:1) to afford the title compound (339 mg, 72%) as a colourless foam; R_f 0.21 (DCM: MeOH 25:1); [α]_D²⁴ +66 (c 0.74, CHCl₃); ν_{max}/cm⁻¹ (CHCl₃) 3603, 3390, 3305, 3200, 2934, 2838, 2552, 1905, 1713, 1681, 1633, 1611, 1584, 1490, 1456; ¹H NMR (400 MHz, chloroform-d) δ 9.49 (1H, s, NH), 9.38 (1H, s, NH), 7.61 (1H, s, C=CHN), 7.46–7.37 (2H, m, Ph), 7.35–7.15 (8H, m, 4 × Ar, C6H, 3 × Ph), 6.86–6.76 (4H, m, Ar), 6.68 (1H, d, J 1.4, C6H), 6.07 (1H, t, J 6.6, C1'H), 5.79 (1H, dd, J 9.7, 2.6, C1'H), 4.72–4.56 (2H, m, NCH₂C), 4.45 (1H, dt, J 7.0, 5.2, C3'H), 4.15–4.00 (2H, m, C4'H and C4'H), 3.80 (1H, d, J 13.9, OC5'HH) 3.78 (6H, s, OCH₃), 3.68 (1H, d, J 13.9, OC5'HH), 3.26 (1H, dd, J 9.7, 5.3, NC5'HH), 3.09–2.97 (2H, m, NC5'HH and NCHH), 2.91 (1H, d, J 10.9, NCHH), 2.36–2.18 (2H, m, C2'H₂), 2.10 (1H, t, 10.7, NCHH), 2.04 (1H, t, 10.7, NCHH), 1.92 (3H, d, J 1.2, CH₃), 1.92 (3H, d, J 1.2, CH₃), 0.89 (9H, s, SiC(CH₃)₃), 0.11 (3H, s, SiCH₃), 0.08 (3H, s, SiCH₃); ¹³C NMR (101 MHz, chloroform-d) δ 163.8 (2 × C4), 158.6 (2 × ^{Ar}C), 150.3 (C2), 150.2 (C2), 144.8 (^{Ar}C), 143.86 (C=) 136.4 (C6H), 135.9 (^{Ar}C), 135.8 (^{Ar}C), 135.62 (C6H), 130.2 (2 × ^{Ar}CH), 130.14 (2 × ^{Ar}CH), 128.23 (2 × ^{Ph}CH), 127.9 (2 × ^{Ph}CH), 126.9 (^{Ph}CH), 124.7 (=CHN), 113.2 (4 × ^{Ar}CH), 111.6 (C5), 110.9 (C5), 86.6 (Ar₃CO), 86.2 (C1'H), 84.2 (C4'H), 79.8 (C1'H), 75.8 (C4'H), 72.0 (C3'H), 64.6 (NC5'H₂), 55.9 (NCH₂), 55.3 (2 × OCH₃), 54.4 (NCH₂), 52.8 (OC5'H₂), 50.9 (NCH₂C), 39.4 (C2'H₂), 25.8 (SiC(CH₃)₃), 17.9 (SiC(CH₃)₃), 12.70 (2 × CH₃), -4.5 (SiCH₃), -4.6 (SiCH₃); HRMS (ESI +ve) C₅₀H₆₃N₈O₁₀Si (M + H⁺) requires 963.4431, found 963.4436, and C₅₀H₆₂N₈NaO₁₀Si (M + Na⁺) requires 985.4250, found 985.4231.

¹¹L-Morpholino-3'-OH dimer T-T (12)

Method 1 - direct Cu^I catalysed cycloaddition with an unprotected azide. To a microwave vial containing the morpholino thymidine (3) (203 mg, 350 μmol) and the azide thymidine (4) (95 mg, 350 μmol) in THF: ^tBuOH: H₂O (3:2:1 ratio, total volume 1.75 mL) was added copper iodide (33.0 mg, 175 μmol). The vial was sealed, stirred and irradiated in a Biotage microwave at 80 °C (approximately power of irradiation 16 W) for 3½ h. After cooling to room temperature the vial was removed, and the solvents were removed *in vacuo* to afford a residue, which was purified by silica gel chromatography, eluting with DCM: MeOH (12:1 to 10:1) to afford the title compound (223 mg, 75%) as a white foam; R_f 0.12 (DCM: MeOH 10:1); [α]_D²⁴ -7.13 (c 1.0, CHCl₃); ν_{max}/cm⁻¹ (ATR) 3390, 3008, 2961, 1690, 1608, 1509, 1490, 1456; ¹H NMR (500 MHz, chloroform-d) δ 9.93 (1H, s, NH), 9.86 (1H, s, NH), 7.72 (1H, s, C=CHN), 7.48–7.41 (2H, m, Ph), 7.37–7.25 (7H, m, 4 × Ar, C6H, 2 × Ph), 7.26–7.18 (1H, m, Ph), 6.95 (1H, s, C6H), 6.89–6.80 (4H, m, Ar), 6.07 (1H, t, J 6.6, C1'H), 5.75 (1H, dd, J 8.8, C1'H), 4.74–4.68 (2H, m, NCH₂C), 4.51 (1H, bs, C3'H),



4.39 (1H, bs, OH), 4.24 (1H, m, C4'H), 4.14–4.05 (1H, m, C4'H), 3.89 (1H, d, J 13.6, OC5'HH), 3.80 (6H, s, $2 \times$ OCH₃), 3.63 (1H, d, J 13.6, OC5'HH), 3.30 (1H, dd, J 9.7, 5.1, NC5'HH), 3.10 (1H, dd, J 9.7, 5.0, NC5'HH), 3.07–2.97 (2H, m, NCHH and NCHH), 2.33–2.26 (2H, m, C2'H₂), 2.18 (1H, t, J 11.0, NCHH), 1.97 (1H, t, J 10.4, NCHH), 1.92 (3H, s, CH₃), 1.89 (3H, s, CH₃); ¹³C NMR (126 MHz, chloroform-*d*) δ 164.0 (C4), 163.9 (C4), 158.5 ($2 \times$ ^{Ar}C), 150.5 (C2), 150.4 (C2), 144.6 (C=), 143.8 (^{Ar}C), 136.6 (C6H), 135.8 (^{Ar}C), 135.7 (^{Ar}C), 135.5 (C6H), 130.1 ($2 \times$ ^{Ar}CH), 130.0 ($2 \times$ ^{Ar}CH), 128.1 ($2 \times$ ^{Ar}CH), 127.8 ($2 \times$ ^{Ar}CH), 126.9 (^{Ph}CH), 124.6 (=CHN), 113.1 ($4 \times$ ^{Ar}CH), 111.3 (C5), 111.0 (C5), 86.52 (C1'H), 86.1 (Ar₃CO), 83.9 (C4'H), 79.9 (C1'H), 75.7 (C4'H), 71.6 (C3'H), 64.3 (NC5'H₂), 55.4 (NCH₂), 55.2 ($2 \times$ OCH₃), 54.9 (NCH₂), 52.7 (OC5'H₂), 51.4 (NCH₂C), 38.9 (C2'H₂), 12.6 (CH₃), 12.5 (CH₃); HRMS (ESI +ve) C₄₄H₄₉N₈O₁₀ (M + H⁺) requires 849.3566, found 849.3644.

Method 2; TBAF deprotection of TBS protected triazole. To a stirred solution of the ^{TL}morpholino-3'-*O*-*tert*-butyl silyl dimer T-T (11) (339 mg, 358 μ mol) in THF (2.0 mL) under an argon atmosphere, was added tetrabutylammonium fluoride trihydrate (141 mg, 447 μ mol). The resulting solution was stirred at room temperature for 12 hours, then ammonium chloride (28 mg, 540 μ mol) was added and stirred for 5 minutes. The resulting reaction mixture was dry loaded on to silica and purified by silica gel chromatography, eluting with DCM:MeOH (12:1 to 10:1) to afford the title compound (251 mg, 82%) as a white foam; R_f 0.12 (DCM:MeOH 10:1).

^{TL}Morpholino phosphoramidite T-T (13)

To a stirred solution of the ^{TL}Morpholino-3-OH dimer T-T (12) in DCM under an argon atmosphere at room temperature was added *N,N*-diisopropylethylamine (98 μ L, 561 μ mol) followed by 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (59.0 mg, 266 μ mol) dropwise over 1 minute, then stirred at room temperature for 24 h. The solvent was blown off with a stream of nitrogen gas, and the residue was purified by silica column chromatography, eluting with DCM:MeOH (20:1 to 15:1) to afford an analytical pure sample of the title compound (38 mg, 14%) as a white foam along with the bulk material (167 mg *ca.* 60%) containing small amounts of 2-cyanoethyl *N,N*-dipropylphosphoramidate as an off white foam; R_f 0.15 (DCM:MeOH 15:1); $\nu_{\max}/\text{cm}^{-1}$ (CHCl₃) 3698, 3665, 3391, 3212, 2857, 2552, 2300, 2105, 2047, 1908, 1731, 1681, 1633, 1592, 1490 and 1455; ¹H NMR (500 MHz, chloroform-*d*) δ 8.66 (1H, s, NH), 8.66 (1H, s, NH), 7.63 and 7.59 (1H, $2 \times$ s, C=CHN), 7.48–7.41 (2H, m, Ph), 7.35–7.23 (7H, m, $4 \times$ Ar, C6H, $2 \times$ Ph), 7.23–7.17 (1H, m, Ph), 6.87–6.77 (5H, m, Ar and C6H), 6.12 (1H, m, C1'H), 5.77 (1H, m, C1'H), 4.82–4.54 (3H, m, NCH₂C and C3'H), 4.34–4.22 (1H, m, C4'H), 4.07–4.02 (1H, m, C4'H), 3.90–3.85 (1H, m, OCHH), 3.84–3.79 (1H, m, OC5'HH), 3.79 (6H, s, $2 \times$ OCH₃), 3.84–3.68 (1H, m, OCHH), 3.69–3.54 (3H, m, OC5'HH and $2 \times$ NCH), 3.26 (1H, dd, J 9.7, 5.1, NC5'HH), 3.08–2.96 (2H, m, NC5'HH and NCHH), 2.92 (1H, m, NCHH), 2.75–2.68 (1H, m, CHHCN), 2.71–2.59 (1H, m, CHHCN), 2.51–2.22 (2H, m, C2'H₂), 2.09 (1H, t, J 11.0, NCHH),

2.07–1.97 (1H, m, NCHH), 1.92 (3H, s, CH₃), 1.90 (3H, s, CH₃), 1.20 (12H, m, NCH(CH₃)₂); ¹³C NMR (500 MHz, chloroform-*d*) due to the presence of a diastereomeric mixture at the phosphorus(III) centre and coupling from phosphorus the ¹³C NMR could not be unambiguously assigned but the spectra are included in this ESI; ³¹P NMR (162 MHz, CDCl₃) δ 149.2; HRMS (ESI +ve) C₅₃H₆₆N₁₀O₁₁P (M + H⁺) requires 1049.4645, found 1049.4653 and C₅₃H₆₅N₁₀NaO₁₁P (M + Na⁺) requires 1071.4464, found 1071.4489.

3'-*O*-Propargyl thymidine (15)

To a stirred solution of 5'-*O*-DMT thymidine (1.40 g, 2.55 mmol) in THF (25 mL) at 0 °C was added sodium hydride (257 mg, 6.4 mmol) in small portions over 5 min. The resulting solution was stirred at 0 °C for 30 min, then at room temp for 1 h. The solution was cooled to 0 °C and propargyl bromide (285 μ L, 3.18 mmol) was added. The solution was stirred at 0 °C for 30 min, then at room temp for 5 h. The reaction was quenched by the addition of water (1 mL), and the volatile organic were removed by evaporation. The residue was partitioned between water (25 mL) and DCM (25 mL), the aqueous layer was extracted with DCM ($3 \times$ 25 mL). The combined organic layers were washed with brine ($2 \times$ 25 mL), dried over MgSO₄, and evaporated *in vacuo* to afford a residue, which was purified by silica gel chromatography, eluting with DCM:MeOH (38:1 to 19:1) to afford the title compound (1.15 g, 77%) as a white foam; R_f 0.4 (DCM:MeOH 19:1); $[\alpha]_{\text{D}}^{24}$ 33.0 (*c* 1.0, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ (ATR); 3385, 3190, 2950, 1930, 1695, 1631, 1611, 1491; ¹H NMR (400 MHz, chloroform-*d*) δ 8.75 (1H, br s, NH), 7.63 (1H, s, C6H), 7.46–7.41 (2H, m, Ar), 7.36–7.25 (7H, m, Ar), 6.89–6.85 (4H, m, Ar), 6.36 (1H, d, J 8.0 and 5.7, C1'H), 4.53 (1H, dt, J 5.3 and 2.2, C4'H), 4.21 (1H, dd, J 15.9 and 2.3, OCHHCCH), 4.21–4.16 (1H, m, C5'H), 4.16 (1H, dd, J 15.9 and 2.3, OCHHCCH), 3.82 (6H, s, $2 \times$ OCH₃), 3.50 (1H, dd, J 10.6 and 3.0, C6'HH), 3.38 (1H, dd, J 10.6 and 2.7, C6'HH), 2.54 (1H, ddd, J 13.9, 5.7 and 2.2, C2'HH), 2.44 (1H, t, J 2.3, CCH), 2.26 (1H, ddd, J 13.9, 8.0 and 6.3, C2'HH), 1.53 (3H, s, CH₃); ¹³C NMR (101 MHz, chloroform-*d*) δ 163.8 (C4), 158.8 ($2 \times$ C), 150.4 (C2), 144.5 (C), 135.6 (C6H), 135.5 ($2 \times$ C), 130.2 ($4 \times$ CH), 128.2 ($2 \times$ CH), 128.1 ($2 \times$ CH), 127.3 (CH), 113.4 ($4 \times$ ArCH), 111.3 (C5H), 87.1 (C), 84.91 (C1'H), 84.0 (C5'H), 79.2 (C), 78.6 (C4'H), 75.2 (CH), 63.6 (C5'H₂), 56.7(CH₂), 55.4 ($2 \times$ OCH₃), 37.9 (C2'H₂), 12.0 (CH₃); HRMS (ESI +ve) C₃₄H₃₄N₂NaO₇ (M + Na⁺) requires 605.2258, found 605.2247.

Triazole-T-T dimer (16)

To a microwave vial containing the 3'-*O*-propargyl thymidine (15) (1.164 g, 2.0 mmol) and the azide thymidine (4) (534 mg, 2.0 mmol) in THF:^tBuOH:H₂O (3:2:1 ratio, total volume 12 mL) was added copper iodide (188 mg, 1.0 mol). The vial was sealed, stirred and irradiated in a Biotage microwave at 80 °C (approximately power of irradiation 16 W) for 3½ h. After cooling to room temperature the vial was removed, and the solvents were removed *in vacuo* to afford a residue, which was purified by silica gel chromatography, eluting with DCM:



MeOH (20 : 1 to 10 : 1) to afford the title compound (1.47 g, 87%) as a white foam; R_f 0.35 (DCM : MeOH 10 : 1); $[\alpha]_D^{24}$ 5.2 (c 1.0, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3392, 3010, 2963, 1691, 1604, 1493, 1460; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.35 (1H, br s, NH), 11.31 (1H, br s, NH), 8.08 (1H, s, triazole-CH), 7.51 (1H, s, C6H), 7.40–7.29 (4H, m, Ar), 7.33 (1H, s, C6H), 7.28–7.22 (5H, m, Ar), 6.90 (4H, d, J 8.7 Hz, Ar), 6.16 (1H, app t, J 6.4 Hz C1H), 6.14 (1H, app t, J 6.4 Hz C1H), 5.50 (1H, d, J 4.4, OH), 4.70 (1H, dd, J 14.3 and 4.4, NC5'HH), 4.59 (1H, dd, J 14.3 and 7.7, NC5'HH), 4.60–4.52 (2H, m, OCH_2C), 4.45–4.37 (1H, m, C3'H), 4.31–4.24 (1H, m, C3'H), 4.10–4.02 (2H, m, C4'H and C4'H), 3.74 (6H, s, $2 \times \text{OCH}_3$), 3.26 (1H, dd, J 10.5, 3.8 Hz, OC5'HH), 3.17 (1H, dd, J 10.5, 3.2 Hz, OC5'HH), 2.43–2.25 (2H, m, C2'HH), 2.25–2.04 (2H, m, C2'HH), 1.78 (3H, s, CH_3), 1.43 (3H, s, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 163.6 ($2 \times \text{C}_4$), 158.17 ($2 \times \text{C}$), 150.37 ($2 \times \text{C}_2$), 144.6 (C), 143.7 (C), 136.0 (C6), 135.5 (C6), 135.4 (C), 135.1 (C), 129.7 ($4 \times \text{CH}$), 127.9 ($2 \times \text{CH}$), 127.6 ($2 \times \text{CH}$), 126.8 (CH), 124.7 (triazole-CH), 113.3 ($4 \times \text{ArCH}$), 109.8 (C5H), 109.7 (C5H), 86.04 (C), 84.02 (CH), 83.93 (CH), 83.78 (CH), 82.87 (CH), 78.75 (CH), 70.72 (CH), 63.77 (CH₂), 61.77 (CH₂), 55.05 ($2 \times \text{OCH}_3$), 51.17 (CH₂), 37.9 (C2'H₂), 36.5 (C2'H₂), 12.1 (CH₃), 11.9 (CH₃); HRMS (ESI +ve) $\text{C}_{44}\text{H}_{47}\text{N}_7\text{NaO}_{11}$ ($\text{M} + \text{Na}^+$) requires 872.3226, found 872.3215.

Triazole-T-T dimer phosphoramidite (17)

To a stirred solution of the triazole-T-T dimer (16) (493 mg, 0.58 mmol) in DCM (1.2 mL) under an argon atmosphere at room temperature was added *N,N*-diisopropylethylamine (182 μL , 1.05 mmol) followed by 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (125 mg, 0.58 mmol) dropwise over 1 minute, then stirred at room temperature for 24 h. The solvent was blown off with a stream of nitrogen gas, and the residue was purified by silica column chromatography, eluting with DCM : MeOH (20 : 1 to 15 : 1) to afford the title compound (391 mg, ca. 64%) containing 2-cyanoethyl *N,N*-diisopropylphosphonamidate as an off white foam; R_f 0.21 (DCM : MeOH 15 : 1); $\nu_{\text{max}}/\text{cm}^{-1}$ (ATR) 3700, 3669, 3384, 3219, 2860, 2550, 2310, 2109, 1911, 1733, 1686, 1495 and 1459; ^1H NMR (500 MHz, chloroform- d) δ 9.20 (2H, br s, NH), 7.69 and 7.65 (1H, $2 \times$ s, triazole-CH), 7.60 (1H, s, C6H), 7.42–7.37 (2H, m, Ar), 7.37–7.21 (7H, m, Ar), 6.90–6.83 (4H, m, Ar), 6.78–6.73 (1H, $2 \times$ s, C6H), 6.38–6.32 (1H, m, C1H), 6.21–6.13 (1H, m, C1H), 4.78–4.57 (5H, m, $2 \times \text{CH}_2$ and OCH), 4.40–4.11 (3H, m, $3 \times \text{CH}$), 3.99–3.70 (2H, m, CH_2), 3.81 (6H, s, $2 \times \text{OCH}_3$), 3.68–3.45 (3H, m, $2 \times \text{CH}$ and CHH), 3.38–3.32 (1H, m, CHH), 2.80–2.75 (1H, m, CHHCN) 2.70–2.65 (1H, m, CHHCN), 2.57–2.20 (4H, m, C2'HH and C2'HH), 1.90–1.87 (3H, m, CH_3), 1.47 (3H, s, CH_3); 1.26–1.18 (12H, m, $\text{NCH}(\text{CH}_3)_2$); δ ^{13}C NMR (500 MHz, chloroform- d) due to the presence of a diastereomeric mixture at the phosphorus(III) centre and coupling from phosphorus the ^{13}C NMR could not be unambiguously assigned but the spectra are included in this ESI; ^{31}P NMR (162 MHz, CDCl_3) δ 149.1; HRMS (ESI +ve) $\text{C}_{53}\text{H}_{65}\text{N}_9\text{O}_{12}\text{P}$ ($\text{M} + \text{H}^+$) requires 1050.4485, found 1050.4464.

Conclusions

We have shown that the CuAAC reaction can be used to synthesise a new DNA mimic containing a triazole-linked morpholino ($^{\text{TM}}$ MO) internucleotide modification. Phosphoramidite reagents 13 and 17 were synthesised and their compatibility with automated solid phase synthesis was demonstrated. UV melting studies showed that incorporation of the $^{\text{TM}}$ MO modification provided an improved T_m value for binding to RNA when compared to the previously reported triazole-containing oligomers. Structural characterisation, and biological evaluation of the $^{\text{TM}}$ MO-modified oligomers is underway and the results of this work will be reported in due course.

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