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Synthesis of triazole-linked morpholino oligonucleotides via CuI catalysed cycloaddition†

Matthew J. Palframan,† Rima D. Alharthy,‡§ Paulina K. Powalowska and Christopher J. Hayes*

Triazole-linked morpholino (TLMO) oligonucleic acids were synthesised using the CuI 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 Tm values than the corresponding TLDNA modification.

Introduction

Click chemistry has recently emerged as a powerful tool in the field of nucleic acid research. In particular, the CuI catalysed (3 + 2) azide–alkyne cycloaddition (CuAAC) has been used to construct modified internucleotide linkages, to prepare nucleic acid conjugates, and as a strand ligation tool. Zerrouki et al., designed a novel triazole-linked DNA analogue (TLDNA) using the CuAAC for oligomer elongation, and this preliminary work has been significantly extended by Brown et al. The artificial TLDNA retains good aqueous solubility, is stable towards enzymatic degradation, and can be read by polymerases, thus making it capable of in vitro transcription and rolling circle amplification. Furthermore, and perhaps most impressively, Brown has demonstrated that genes containing TLDNA are functional in vivo in Escherichia coli and in human cells. Given the biocompatibility of the TLDNA 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. 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 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 as they could combine the ease of synthesis of the TLDNAs with the increased melting temperatures associated with morpholino drug candidates. The TLMO hybrid can be disconnected to reveal the azide and the alkyne-substituted morpholine as potential precursors for the proposed CuAAC reaction (Fig. 1).

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

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 : BuOH : H2O (3:2:1) with microwave heating (80 °C) was optimal (Scheme 3). Under these conditions, cycloaddition of the acetylene 3 with the TBS-protected azide 10 gave the triazole-linked morpholino (TLMO) 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 TLMO 12 was converted to the 3′-cyanoethyl phosphoramidite 13 (74%) under standard conditions (Scheme 3).

To provide a direct comparison of the new TLMO hybrid 2 to the triazole-linked DNA analogue (TLDNA) 1, we next prepared the phosphoramidites 13. 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 TLMO-containing oligomer 21 and the known TLDNA oligomer 2210 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 complimentary DNA strand 18,10 we also prepared 18 so that we could directly compare the Tm values of 18 + 21, 18 + 22 and the unmodified duplex (18 + 20) under the same conditions. In order to assess the potential
use of the TLMO-modification in therapeutically useful oligomers, we also synthesised the complimentary RNA oligonucleotide, as this simulates an intracellular mRNA target. The integrity of the oligomers was confirmed by ESI mass spectrometry (Table 1) and HPLC (see ESI†).

Thermal stabilities of the TLMO, TLDNA, and unmodified DNA duplexed with complimentary DNA (Fig. 2) and RNA (Fig. 3) were then determined by UV melting experiments15 (Table 2). Pleasingly, the $T_m$ values of the control DNA: DNA (62.4 °C) (entry 1, Table 2), and the DNA: TLDNA (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 TLMO-containing oligomer duplexed to DNA gave a $T_m$ value of 56.1 °C (entry 2, Table 2), which represents a small increase ($\Delta T_m$ 1.0 °C) over that determined for 22, but still represents a significant decrease from the unmodified DNA ($\Delta T_m$ −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 duplexed with unmodified DNA was determined (58.5 °C) as a control (entry 4, Table 2). In contrast to the duplexes with DNA, the $T_m$ of TLMO (56.6 °C) was much closer to that of the unmodified DNA: RNA than was TLDNA (54.1 °C) with RNA (Δ$T_m$ −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 TLMO-modified oligomers, before selecting the best candidates for biological evaluation.

Scheme 4  Synthesis of 3′-cyanoethyl phosphoramidite 17.

![Scheme 4](image)

Table 1  Sequences of oligonucleotides synthesised

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Sequence 5′ → 3′</th>
<th>m/z Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 (DNA)</td>
<td>d(GCTGCAACGTGC)</td>
<td>3953.55</td>
<td>3953.73</td>
</tr>
<tr>
<td>19 (RNA)</td>
<td>GCUGCAACGUCG</td>
<td>4133.49</td>
<td>4133.63</td>
</tr>
<tr>
<td>20</td>
<td>d(CGACGTTTCGAC)</td>
<td>3944.53</td>
<td>3944.72</td>
</tr>
<tr>
<td>21</td>
<td>d(CGACGTTCGACG)</td>
<td>3944.64</td>
<td>3944.80</td>
</tr>
<tr>
<td>22</td>
<td>d(CGACGTTCGACG)</td>
<td>3945.63</td>
<td>3945.78</td>
</tr>
</tbody>
</table>

* T indicates the position of the morpholine-triazole modification.  
** T indicates the position of the triazole modification.

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

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

* $T_m$ values for 3 µM oligo samples. Values in parentheses refer to cooling curves.  
** $\Delta T_m$ per modification relative to the control DNA 20.
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 biorbate (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); Rf 0.28 (CHCl3 : MeOH, 25 : 1); [α]D25 +47 (c 0.61, CHCl3); νmax/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, CH6), 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, ddt, 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′H, C2′H), 1.95 (3H, s, CH3); 13C NMR (100 MHz, CDCl3) 164.1 (C), 155.8 (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), 86.1 (C), 80.5 (CH), 78.0 (CH), 64.5 (CH2), 55.2 (CH2), 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 compounds were removed by vacuum distillation. 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; Rf 0.17 (DCM : MeOH 25 : 1); [α]D25 +30 (c 0.93, CHCl3); νmax/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, C6F6), 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=CHH), 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′H3H6), 2.84 (1H, br d, J 11.4, C2′HH), 2.32 (1H, t, J 2.3, C=C=H), 2.34–2.29 (2H, m, C3′H′, 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 (C), 75.7 (CH), 74.5 (CH), 64.6 (CH2), 55.3 (CH3), 54.6 (CH2), 52.8 (CH2), 46.4 (CH2), 17.9 (CH3); HRMS (ESI) C34H36N3O6 (M + H+) requires 582.2599, found 582.2569.

5′-O-Mesyf-3′-O-tert-butylidemethylsilyl deoxythymidine (9)

To a stirred solution of 5′-OH-3′-O-tert-butylidemethylsilyl 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 (2.0 g, Quant.) as a yellow foam, which was used without further purification; νmax/cm−1 (CHCl3) 3393, 3006, 2955, 2930, 2885, 1690, 1471, 1362, 1230, 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), 4.45 (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, OCH3), 2.28 (1H, ddt, 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 (C6H5), 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 ESL.†
(SiCH₃)₃, -4.8 (SiCH₃); HRMS (ESI) C₁₂H₂₃N₂O₂SSi (M + H) requires 435.1616; found 436.1624 and C₁₂H₂₀N₂NaO₂SSi (M + Na) requires 457.1435; found 457.1444.

5′-Azido-3′-O-tert-butyldimethylsilyl deoxythymidine (10)

A solution of 5′-O-mesy1-3′-O-tert-butyldimethylsilyl 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 ºC 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 ether (1 : 1 to 0 : 1) to afford a white foam; Rf 0.18 (petrol : diethyl ether 1 : 1)

[α]D +80 (c 0.78, CHCl₃); υmax/cm⁻¹ [CHCl₃] 3030, 3309, 3305, 3200, 2934, 2838, 2552, 1905, 1713, 1681, 1633, 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-C=CH), 7.46–7.37 (2H, m, Ph), 7.35–7.15 (8H, m, 4 × Ar, C₆H₃, 3 × Ph), 6.86–6.76 (4H, m, Ar), 6.68 (1H, d, J 1.4, C₆H₃), 6.07 (1H, t, 10.6, C₆H₃), 5.79 (1H, dd, J 9.7, 2.6, C₆H₃), 5.47–5.36 (2H, m, NCH₂C), 4.45 (1H, dt, J 7.0, 5.2, C₃H), 3.13–3.00 (2H, m, NCH₂C), 2.65 (2H, d, J 13.9, C₆H₃), 2.30 (1H, dd, J 9.7, 5.3, C₅H₅N), 2.09–2.00 (2H, m, N(CH₂CH₃)₂), 1.92 (3H, d, J 13.6, CH₃), 0.89 (9H, s, Si(CH₃)₃), 0.11 (3H, s, SiCH₃), 0.08 (3H, s, SiCH₃); ¹³C NMR (101 MHz, chloroform-d) δ 163.8 (2 × C), 158.6 (2 × NC₅), 150.3 (C₂), 150.2 (C₂), 144.8 (4 × C =), 143.8 (C=C) 136.4 (C₆H₅), 135.9 (NC₅), 135.8 (NC₆), 135.6 (C₆H), 130.2 (2 × NC₆H), 130.14 (2 × NC₆H₃), 128.3 (2 × PhC), 127.9 (2 × PhC), 126.9 (PhC₃), 124.7 (≡CH), 113.2 (4 × PhC ≡CH), 111.6 (C₃), 110.9 (C₅), 86.6 (ArC), 84.2 (C₆H₄), 79.8 (C₁H₇), 75.8 (C₆H₅), 72.0 (C₃_H), 64.6 (NC₅H₅), 55.9 (NCH₃), 55.3 (2 × OCH₃), 54.4 (NCH₂), 52.8 (OC₅H₅), 50.9 (NCH₃C), 39.4 (C₂H₃), 25.8 (SiCH₃), 17.9 (Si(CH₃)₂), 12.70 (2 × CH₃), -4.54 (SiCH₃), -4.77 (SiCH₃); HRMS (ESI +ve) C₅₀H₆₃N₈O₁₀Si (M + H+) requires 963.4431, found 963.4436, and C₂₀H₂₃N₇NaO₁₀Si (M + Na⁺) requires 985.4250, found 985.4231.

1′-Morpholin-3′-O-tert-butyl silyl deoxythymidine (11)

To a stirred solution of 5′-azido-3′-O-tert-butyldimethylsilyl deoxythymidine (10) (151 mg, 400 μmol) in THF (2.0 mL) under argon atmosphere, was added tetraethylammonium 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 : CHCl₃ (1 : 1) to afford the title compound (95 mg, 89% as white foam); Rf 0.20 (EtOAc); [α]D +114 (c 1.0, CHCl₃); υmax/cm⁻¹ (CHCl₃) 3390, 3009, 2956, 2150, 1690, 1471, 1438, 1262; ¹H NMR (500 MHz, Methanol-d₄) δ 7.54 (1H, q, J 1.2, C₆H₃), 6.26 (1H, t, J 6.0, C₁H₇), 4.34 (1H, dt, J 6.5, 4.1, C₃H₃), 3.96 1H, (d, J 5.0, 3.8, C₅H₃), 3.63 (1H, dd, J 13.2, 3.7, C₅H₃), 3.57 (1H, dd, J 13.2, 5.1, C₅H₃), 2.31 (1H, dd, J 13.7, 6.6, C₂H₂), 2.25 (1H, d, d, J 13.7, 6.6, 3.9, C₂H₂), 1.89 (3H, d, J 13.1, CH₃); ¹³C NMR (126 MHz, Methanol-d₄) δ 166.3 (C₄), 152.3 (C₂), 137.7 (C₆H), 111.9 (C₅), 86.4 (C₆H₄ or C₁H₇) 86.3 (C₄H₄ or C₁H₇), 72.5 (C₃H), 53.4 (C₆H₆), 40.2 (C₂H₂), 12.5 (CH₃); HRMS (ESI) C₁₉H₁₄N₄O₄ (M + H) requires 268.1040; found 268.1044 and C₁₉H₁₄N₄NaO₄ (M + Na) requires 290.0859; found 290.0858.

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CHHCN), 2.51

13C NMR (126 MHz, chloroform-d) δ 164.0 (C4), 163.9 (C4), 158.5 (2 × ArC), 150.3 (C2), 150.4 (C2), 144.6 (C=), 143.8 (N′C), 136.6 (C6H), 133.8 (N′C), 135.7 (C′C), 135.5 (C6H), 130.1 (2 × ArCH), 130.0 (2 × ArCH), 128.1 (2 × ArCH), 127.8 (2 × ArCH), 126.9 (PhCH), 121.6 (C′H), 64.3 (N′C5′H), 55.4 (NCH2), 55.2 (2 × OCH3), 54.9 (NCH2), 52.7 (OC5′H), 51.4 (NCH2C), 38.9 (C′H22), 12.6 (CH3), 12.5 (CH3); HRMS (ESI +ve) C54H49N8O10 (M + H+).

Method 2: TBAF deprotection of TBS protected triazole. To a stirred solution of the Tmorpholino-3′-O-tert-butyl silyl dimer T-T (11) (339 mg, 358 μmol) in THF (2.0 mL) under an argon atmosphere, was added tetrabutylammonium fluoride in DCM under an argon atmosphere at room temperature was added tetrabutylammonium fluoride trihydrate (141 mg, 447 μmol). The resulting solution was stirred at room temperature for 12 hours, then ammonium chloride (28 mg, 540 μmol) 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; Rf 0.12 (DCM : MeOH 10 : 1).

Tmorpholino phosphoramidite T-T (13)

To a stirred solution of the Tmorpholino-3′-OH dimer T-T (12) in DCM under an argon atmosphere at room temperature was added N,N-diisopropylethylamine (98 μL, 561 μmol) followed by 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (59.0 mg, 266 μmol) 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; Rf 0.15 (DCM : MeOH 15 : 1); νmax/cm−1 (CHCl3) 3698, 3665, 3391, 3212, 2857, 2552, 2300, 2105, 2047, 1908, 1731, 1681, 1633, 1592, 1490 and 1455; 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, C′H), 4.53 (1H, dt, J = 5.3 and 2.2, C′H), 4.21 (1H, dd, J = 15.9 and 2.3, OCHCHCH), 4.21–4.16 (1H, m, C5′H), 4.16 (1H, dd, J = 15.9 and 2.3, OCHCHCH), 3.82 (6H, s, 2 × OCH3), 3.50 (1H, dd, J = 10.6 and 3.0, C′6H), 3.38 (1H, dd, J = 10.6 and 2.7, C′6H), 2.54 (1H, ddd, J = 13.9, 5.7 and 2.2, C2′H2), 2.44 (1H, t, J = 2.3, C′H), 2.26 (1H, ddd, J = 13.9, 8.0 and 6.3, C2′H2), 1.53 (3H, s, CH3); 13C NMR (101 MHz, chloroform-d) δ 163.8 (C4), 158.8 (2 × C), 150.4 (C2), 144.5 (C), 135.6 (C6H), 135.2 (2 × C), 130.2 (4 × CH), 128.2 (2 × CH), 128.1 (2 × CH), 127.3 (CH3), 113.4 (4 × ArCH), 111.3 (C5H), 87.1 (C), 84.91 (C′H), 84.0 (C′6H), 79.2 (C), 78.6 (C′6H), 75.2 (CH), 63.6 (C5′H), 56.7(CH3), 55.4 (2 × OCH3), 37.9 (C2′H2), 12.0 (CH3); HRMS (ESI +ve) C54H49N8O10 (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:BuOH: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 32 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); \( \delta \text{H} (\text{DMSO-}d_6) 5.2 \) (c 1.0, CHCl_3); \( \nu_{\text{max}}/\text{cm}^{-1} \) (ATR) 3392, 3010, 2963, 1691, 1604, 1493, 1460; \(^1\text{H} \) NMR (400 MHz, DMF-\text{d}_6) \( \delta 11.35 \) (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\'\text{HH}), 4.59 (1H, dd, J 14.3 and 7.7, NC5\'\text{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 OCH_3), 3.26 (1H, dd, J 10.5, 3.8 Hz, OC5\'\text{HH}), 3.17 (1H, dd, J 10.5, 3.2 Hz, OC5\'\text{HH}), 2.43–2.25 (2H, m, C2\'\text{HH}), 2.25–2.04 (2H, m, C2\'\text{HH}), 1.78 (3H, s, CH_3), 1.43 (3H, s, CH_3); \(^{13}\text{C} \) NMR (101 MHz, DMSO) \( \delta 163.6 \) (2 × C4), 158.17 (2 × C), 150.37 (2 × C2), 144.6 (C), 143.7 (C), 136.0 (C6), 135.5 (C6), 135.4 (C6), 135.1 (C), 129.7 (4 × C), 127.9 (2 × CH), 127.6 (2 × CH), 126.8 (CH), 124.7 (triazole-CH), 113.3 (4 × ArCH), 109.8 (C5H), 109.7 (C5H), 86.04 (C), 84.02 (CH3), 83.93 (CH), 83.78 (CH), 82.87 (CH), 78.75 (CH), 70.72 (CH), 67.37 (CH2), 61.77 (CH2), 55.05 (2 × OCH3), 51.17 (CH2), 37.9 (C2\'H2), 36.5 (C2\'H2), 12.1 (CH3), 11.9 (CH3); HRMS (ESI +ve) \( C_{91}H_{97}N_7NaO_{11} \) (M + Na\(^{+}\)) 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 (TMOM) 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 TMOM modification provided an improved Tm value for binding to RNA when compared to the previously reported triazole-containing oligomers. Structural characterisation, and biological evaluation of the TMOM-modified oligomers is underway and the results of this work will be reported in due course.

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Notes and references