Bioorganic & Medicinal Chemistry 19 (2011) 1162–1166

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and triplex-forming ability of oligonucleotides bearing 1-substituted 1*H*-1,2,3-triazole nucleobases

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ARTICLE INFO

Article history:
Received 22 November 2010
Revised 22 December 2010
Accepted 23 December 2010
Available online 30 December 2010

Keywords:
Oligonucleotide
Synthesis
Click chemistry
Triple helix
CG recognition

ABSTRACT

Using the copper(I)-catalyzed alkyne-azide 1,3-dipolar cycloaddition, a post-elongation modification of 1-ethynyl substituted nucleobases has been employed to construct 18 variations of oligonucleotides from a common oligonucleotide precursor. The triplex-forming ability of each oligonucleotide with dsDNA was evaluated by the UV melting experiment. It was found that triazole nucleobases generally tend to exhibit binding affinities in the following order: CG > TA > AT, GC base pairs. Among the triazole nucleobases examined, a 1-(4-ureidophenyl)triazole provided the best result with regard to affinity and selectivity for the CG base pair.

1. Introduction

Triplex formation of double-stranded DNA (dsDNA) and an oligonucleotide is a foundation of nucleic acid-based technologies such as gene therapy and gene diagnosis.[1] However, natural oligonucleotides bind only to the homopurine strand of the homopurine–homopyrimidine tract within dsDNA, forming triplex DNA through either the Hoogsteen hydrogen bonds or the reverse-Hoogsteen interactions. In other words, no natural nucleobase can form a base triplet with a pyrimidine–purine (CG or TA) interruption within a homopurine–homopyrimidine tract of dsDNA. The development of artificial nucleic acids, particularly artificial nucleobases capable of recognizing CG or TA base pairs, has been explored to address this issue.[2,3] The development of a more efficiently recognizable nucleobase remains a challenge and is currently in great demand.

We have also engaged in the development of a nucleic acid combined with 2′,4′-BNA (2′,4′-bridged nucleic acid) modification and nucleobases which sequence-selective and strongly recognize CG or TA base pairs.[4–6] As previously demonstrated by our group, 2′,4′-BNA bearing an oxazol-4-yl nucleobase (O^B) recognizes the CG base pair with a slight sequence-selectivity. However, the BNA–imidazol-1-yl analog (I^B) exhibited the least affinity for the CG base pairs among all of the natural base pairs. This indicates that a hydrogen-acceptor at the α-position of glycosidic bond could be vital for CG base pair recognition as depicted in Figure 1.[4]

In addition, we have recently reported the facile synthesis and the respective duplex-forming abilities of several oligonucleotides, including 2′-deoxyribonucleotides, having 1-substituted 1,2,3-triazol-4-yl nucleobases. The chemical syntheses of these compounds were effected by implementation of the post-elongation modification (PEM) using the copper(I)-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) reaction.[7,8] The PEM method facilitates the preparation of oligonucleotides bearing various 1-substituted 1,2,3-triazol-4-yl nucleobases from a common oligonucleotide precursor in only one synthetic step (i.e., CuAAC reaction). Moreover, the triazole nucleobases have a nitrogen atom at the α-position of the glycosidic bond, which may be necessary for CG base pair recognition. Thus, we set out to explore the affinities and selectivities of nucleobases bearing a triazole core with the aim of finding a nucleobase that effectively recognizes CG base pairs. Here we

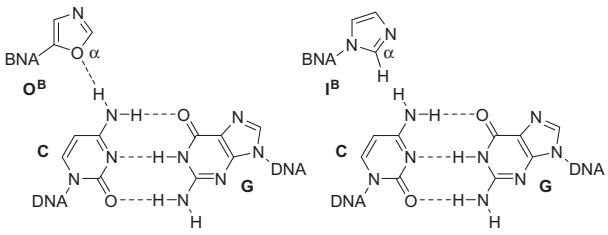

Figure 1. Plausible structures of O^B-CG and I^B-CG base triplets.

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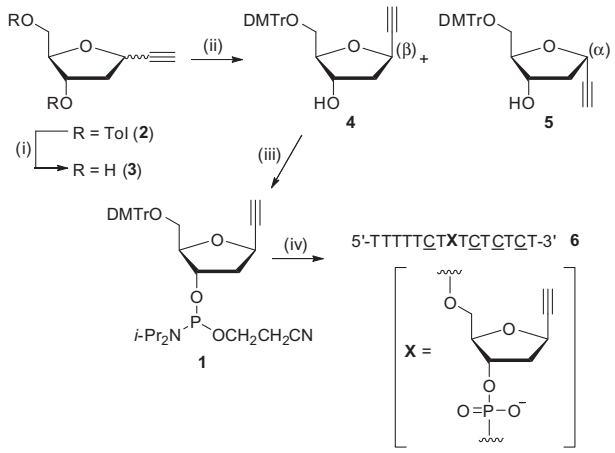
doi:10.1016/j.bmc.2010.12.049

report our successful studies toward this goal culminating in the synthesis of oligonucleotides bearing a wider variety of 1-substituted 1,2,3-triazole nucleobases and their subsequent evaluation of base pair recognition in the formation of triplex DNA. The details of these results are described in this work.[9]

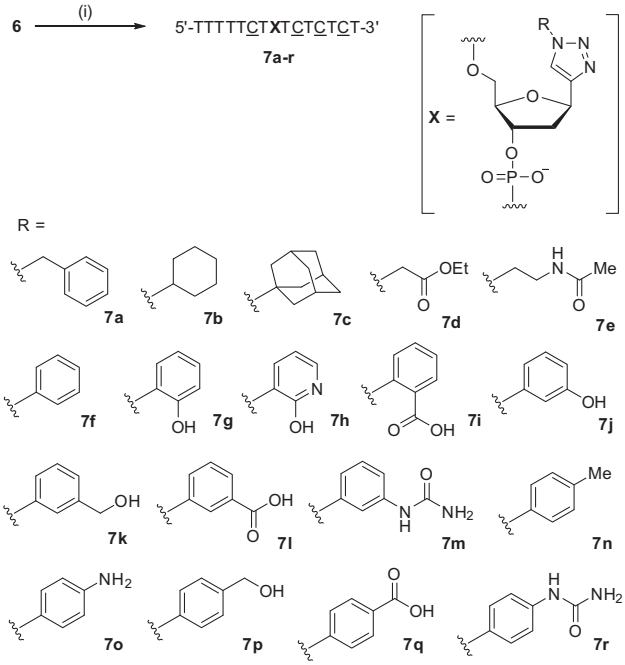
2. Results and discussion

The phosphoramidite **1** was synthesized as depicted in Scheme 1. Methanolysis of the anomeric mixture of toluoyl esters **2** (α:β = ca. 3:1) afforded the diol **3**, which was treated with DMTrCl in pyridine to afford **4** and **5** chemoselectively. At this point, the anomeric mixture could be separated by silica gel column chromatography to provide the desired β-isomer **4** (18% yield) and the α-isomer **5** (56% yield). Phosphitylation of **4** provided the desired phosphoramidite **1**, which was subsequently employed in an oligonucleotide synthesis using an automated DNA synthesizer to give the oligonucleotide **6**. Next, the construction of triazole nucleobases was examined according to our previously optimized CuAAC conditions.[9] The triplex-forming oligonucleotides (TFOs) that were prepared (**7a–r**) are depicted in Scheme 2 (see experimental for details).[10]

The triplex-forming ability of **7a–r** bearing triazole nucleobases with dsDNA targets (XY = CG, TA, GC or AT), was evaluated by the UV melting experiment under neutral conditions [7 mM phosphate buffer (pH 7.0), 140 mM KCl and 10 mM $MgCl_2$]. These results are summarized in Table 1. When substituents at the 1-position of the triazole nucleobases were aliphatic groups, such as benzyl (**7a**), cyclohexyl (**7b**), adamantyl (**7c**), (ethoxycarbonyl)methyl (**7d**) or 2-(acetamido)ethyl (**7e**), the T_m values with dsDNA (XY = CG) ranged from 17 °C to 20 °C. Athough this interaction occurred with selectivity for the CG base pair, its magnitude is less than that of natural TFO **8** (25 °C) with dsDNA (XY = CG).[11] However, even TFO **7c**, containing a bulky adamantyl group, was observed to form the triplexes. The installation of an additional functional group (e.g., **7d** and **7e**) allowed for the formation of hydrogen bonds, resulting in the loss of selectivity for the CG base pair caused by an increase in affinity for the TA base pair. The TFOs **7f–r** contain the aryl-substituted triazole nucleobases. Interestingly, the phenyltriazole nucleobase **7f** led to a significant increase in the affinity to CG and TA base pairs as compared to that observed with the aliphatic-substituted triazoles **7a–e**. This is presumably due to the reduction of entropic penalty for base pair-recognition by the conformational rigidity of the phenyl substituent and an increase in the stability effected by additional π-stacking interactions. The T_m value observed with the triplex dsDNA complex (XY = CG) and the T_m difference (ΔT_m), were 25 °C and 8 °C, respectively. The former was analogous to that of a triplex containing a T-CG base triplet formed by TFO **8** and dsDNA (XY = CG). Previously, it was reported by Dervan's group that the binding affinity of the 4-phenylimidazole nucleobase (D2) to all four base pairs was very weak (Fig. 2a).[12] Therefore, it was suggested that a nitrogen atom at the α-position (e.g., triazole nucleobases) of the glycosidic bond could form a hydrogen bond with the 4-amino group of **C**. The



Scheme 1. Reagents and conditions: (i) NaOMe, MeOH, rt, 3 h, 92%; (ii) DMTrCl (1.5 equiv), pyridine, rt, 2 h, 18% for **4** and 56% for **5**; (iii) $(i\text{-}Pr_2N)_2POCH_2CH_2CN$ (1.5 equiv), diisopropylammonium tetrazolide (1.0 equiv), MeCN–THF (3:1), rt, 4 h, 76%; (iv) DNA synthesis (C = 2′-deoxy-5-methylcytidine).



Scheme 2. Reagents and conditions: (i) R–N_3 (10 equiv), $CuSO_4$ (2 equiv), sodium ascorbate (4–20 equiv), tris[(1-benzyl-1,2,3-triazol-4-yl)methyl]amine (TBTA) (4 equiv), DMSO-phosphate buffer (10 mM, pH 7.0), rt, 1–24 h.

Table 1
T_m values (°C) of triplexes between TFOs **7** and four dsDNA targets[a]

TFO	XY			
	CG	TA	GC	AT
T (8)[b]	25	17	20	44
7a	19	11	<10	<10
7b	20	12	<10	<10
7c	17	11	<10	<10
7d	20	14	12	<10
7e	20	14	<10	14
7f	25	17	<10	<10
7g	25	15	<10	17
7h	24	15	15	12
7i	17	15	12	<10
7j	21	14	<10	<10
7k	23	14	<10	<10
7l	22	15	<10	<10
7m	22	17	<10	13
7n	21	13	<10	<10
7o	23	18	<10	12
7p	24	16	<10	<10
7q	21	<10	<10	<10
7r	24	13	13	12

[a] Conditions: 7 mM sodium phosphate buffer (pH 7.0), 140 mM KCl and 10 mM $MgCl_2$. The final concentration of each oligonucleotide used was 1.5 μM. The sequence of dsDNA targets is as follows: 5′-GCTAAAAAGAXAGAGAGATCG-3′/5′-CGATCTCTCTYTCTTTTTAGC-3′ (XY = CG, TA, GC or AT).

[b] The sequence of TFO **8** is 5′-TTTTTCTTTCTCTCT-3′.

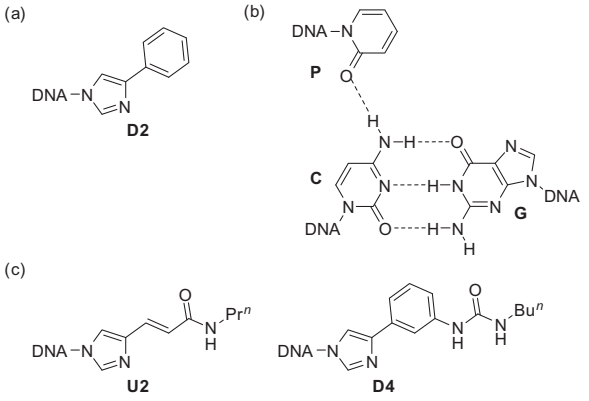


Figure 2. (a) The structure of D2. (b) The structure of a P-CG base triplet. (c) The structures of U2 and D4.

formation of triplexes was further observed with dsDNA bearing purine–pyrimidine base pairs (XY = GC or AT) with the TFOs **7g** and **7h** possessing an *o*-hydroxy phenyl or a 2-hydroxypyridin-3-yl group. On the contrary, a carboxyl group (e.g., **7i**) at the *o*-position led to a dramatic decrease in the T_m value with dsDNA (XY = CG) as compared to that of the unsubstituted TFO **7f**; this is most likely due to the increase in steric bulk imposed by the *ortho* substituent. *Meta* substitution on the phenyl group (e.g., **7j**–**m**) led to somewhat decreased affinities for CG and TA base pairs. A *para* methyl substituent (i.e., **7n**) decreased both affinities to CG and TA base pairs by 4 °C as compared with those of the unsubstituted phenyl TFO **7f**. An additional effect of an amino (i.e., **7o**) or a hydroxymethyl (i.e., **7p**) group on base pair recognition ability was not observed. Interestingly, the *p*-carboxyl substituent in **7q** led to a greater decrease of the T_m value with dsDNA (XY = TA) than that observed with dsDNA (XY = CG). Consequently, the ΔT_m was greater than 11 °C, although the T_m value with dsDNA (XY = CG) was not sufficiently large. Gratifyingly, the TFO **7r** bearing a *p*-ureido group was found to exhibit high sequence-selectivity without the loss of the affinity for dsDNA (XY = CG). The T_m and ΔT_m of the TFO **7r** dsDNA complex (XY = CG) was 24 °C and 11 °C, respectively. This result is comparable to that of the 2-pyridone nucleobase CG base complex (Fig. 2b).[13]

The triplex-forming ability of **7r** was evaluated and compared to those of unsubstituted **7f** and natural **8** (Table 2 and Figure 3) under different conditions, that is, 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM $MgCl_2$. Analogous to the results obtained in phosphate buffer shown in Table 1, the TFO **7r** exhibited high sequence-selectivity with dsDNA containing CG base pairs (Fig. 4). The T_m value of **7r** (29 °C) was comparable with those observed with the dsDNA complexes (XY = CG) with **7f** and **8**. The results provided from the TFOs **7f** and **7r** indicate that a *p*-ureido group decreases only the affinity for TA base pairs;

Table 2
T_m values (°C) of triplexes between TFOs **7f** and 7**r**, and four dsDNA targets[a]

TFO	XY			
	CG	TA	GC	AT
T (**8**)[b]	28	21	22	45
7f	29	23	17	19
7r	29	18	17	18

[a] Conditions: 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM $MgCl_2$. The final concentration of each oligonucleotide used was 1.9 μM. The sequence of dsDNA targets is as follows: 5′-GCTAAAAAGAXAGAGAGATCG-3′/5′-CGATCTCTCTYTCTTTTTAGC-3′ (XY = CG, TA, GC or AT).
[b] The sequence of TFO **8** is 5′-TTTTTCTTTCTCTCT-3′.

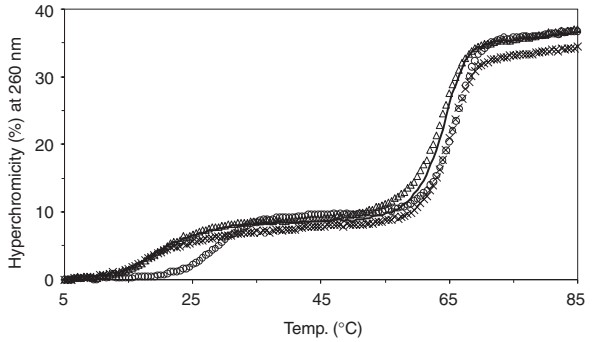


Figure 3. UV melting profiles for triplexes between TFO **7r** and dsDNAs (XY = CG, circle; XY = TA, triangle; XY = GC, cross; XY = AT, line) as shown in Table 2. The presented melting curves were normalized.

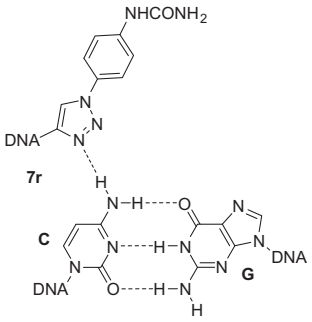


Figure 4. Plausible structure of a **7r**-CG base triplet.

however, detailed explanation of this observation requires further experimentation.

Among five-membered heteroaromatic-containing nucleobases (e.g., U2[14] and D4[15] shown in Fig. 2c) developed for CG base pairs, the 1-(4-ureidophenyl)triazole nucleobase could be a promising candidate, although further improvements in stability and selectivity are required. Furthermore, our present work demonstrates that distal substituents on the triazole core (i.e., ureido moiety) in **7r** can significantly affect the base pair recognition ability and/or sequence-selectivity.

3. Conclusion

An oligonucleotide including an ethynyl moiety was efficiently synthesized and subsequently converted to a wide variety of 1-substituted triazole derivatives by employment of the CuAAC reaction as a PEM method. The triazole nucleobases within oligonucleotides exhibited a significant binding affinity for CG base pairs in a sequence-selective fashion. Among the substrates tested, it was found that the 1-(4-ureidophenyl)triazole nucleobase provided the best result with regard to the binding affinity and CG sequence-selectivity. Moreover, the relationship between the structure and base-pair recognition obtained in this study could provide additional insight into the design of nucleobases containing a five-membered heteroaromatic core to provide more effective CG base pair ligands.

4. Experimental

4.1. General

All chemicals were purchased from chemical suppliers. For column chromatography, Fuji Silysia silica gel PSQ-100B and FL-100D

were used. All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a JEOL ECS400 spectrometer. IR spectra were recorded on JASCO FT/IR-200 and JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO DIP-370 instrument. Mass spectra were measured on a JEOL JMS-600 or JEOL JMS-700 mass spectrometer. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex II TOF/TOF mass spectrometer. EYELA Cute Mixer CM-1000 was used as a shaker.

4.1.1. 1-Ethynyl-2-deoxy-D-ribose (3)

NaOMe (521 mg, 9.64 mmol) was added to a solution of **2**[16] in MeOH (30 mL) at room temperature and the mixture was stirred for 3 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to give compound **3**[17] (α:β = 3:1).

4.1.2. 1-(β)-Ethynyl-5-*O*-(4,4′-dimethoxytrityl)-2-deoxy-D-ribose (4) and 1-(α)-ethynyl-5-*O*-(4,4′-dimethoxytrityl)-2-deoxy-D-ribose (5)

Under a nitrogen atmosphere, DMTrCl (1.21 g, 2.95 mmol) was added to a solution of compound **3** (α:β = 3:1, 280 mg, 1.97 mmol) in anhydrous pyridine (60 mL) at room temperature and the mixture was stirred for 2 h. After addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with AcOEt. The organic extracts were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (*n*-hexane/AcOEt = 4:1) to give compound **4** (159 mg, 18%) and **5** (461 mg, 56%). Compound **4**[18]: $[\alpha]_D^{26}$ 24.4 (*c* 1.03, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.80 (1H, d, *J* = 3.3 Hz), 2.15 (1H, ddd, *J* = 3.0, 6.6 and 17.2 Hz), 2.24 (1H, ddd, *J* = 6.0, 8.7 and 17.2 Hz), 2.46 (1H, d, *J* = 2.1 Hz), 3.15 (1H, dd, *J* = 6.0 and 12.8 Hz), 3.26 (1H, dd, *J* = 4.5 and 12.8 Hz), 3.78 (6H, s), 3.86–3.91 (1H, m), 4.35–4.38 (1H, m), 4.76 (1H, m), 6.78–6.83 (4H, m), 7.16–7.69 (9H, m). Compound **5**: $[\alpha]_D^{25}$ 0.926 (c 0.98, $CHCl_3$). IR ν_{max} (KBr) 3280, 2934, 2044, 1611, 1509, 1251, 1221, 1177, 1085, 1034 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.07 (1H, dt, *J* = 3.6 and 13.4 Hz), 2.11 (1H, d, *J* = 6.7 Hz), 2.45 (1H, ddd, 6.7, 7.9 and 13.4 Hz), 2.51 (1H, d, *J* = 1.8 Hz), 3.10 (1H, dd, *J* = 5.5 and 9.8 Hz), 3.16 (1H, dd, *J* = 4.3 and 9.8 Hz), 3.72 (6H, s), 4.09–4.12 (1H, m), 4.21–4.25 (1H, m), 4.80–4.85 (1H, m), 6.78–6.83 (4H, m), 7.12–7.40 (9H, m). ^{13}C NMR ($CDCl_3$) δ 41.8, 55.2, 64.0, 67.3, 73.9, 74.6, 84.0, 86.2, 113.1, 126.8, 127.8, 128.0, 130.0, 135.8, 144.7, 158.4. HRMS (FAB) *m/z* calcd for $C_{28}H_{28}NaO_5$ $[M+Na]^+$: 467.1834; found, 467.1840.

4.1.3. 1-(β)-Ethynyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-deoxy-D-ribose (1)

Under a nitrogen atmosphere, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (0.10 mL, 0.33 mmol) was added to a solution of compound **4** (77 mg, 0.17 mmol) and diisopropylammonium tetrazolide (35 mg, 0.17 mmol) in anhydrous MeCN–THF (3:1, 2.4 mL) at room temperature and the mixture was stirred for 4 h. The solvent was removed under reduced pressure and the residue was purified by flash silica gel column chromatography (*n*-hexane/AcOEt = 5:1) to give compound **1**[19] (80 mg, 69%) as a colorless oil. ^{31}P NMR ($CDCl_3$) δ 148.5, 149.1.

4.2. Oligonucleotide synthesis

The synthesis of **6** was performed on a 0.2-μmol scale on an automated DNA synthesizer (Applied Biosystems Expedite™ 8909) using the common phosphoramidite protocol except for a prolonged coupling time of 5 min for unnatural phosphoramidites. TFOs synthesized on DMTr-ON mode were cleaved from the CPG resin by treatment with 28% aqueous NH_3 solution at room temperature for 1.5 h. Additional treatment with 28% aqueous NH_3 solution at 55 °C for 15 h underwent the removal of all protecting groups on TFOs. The obtained crude TFOs were purified with Sep-Pak® Plus C18 cartridges (Waters) followed by reversed-phase HPLC (Waters XTerra® MS C_{18} 2.5 μm, 10 × 50 mm). The composition of the TFOs was confirmed by MALDI-TOF-MS analysis. MALDI-TOF-MS data ($[M-H]^-$) for **6**: found 4395.39 (calcd 4395.91).

4.3. Azide synthesis

Among azide reagents used for click chemistry, 1-(3-azidophenyl)urea and 1-(4-azidophenyl)urea were new compounds which were prepared according to the following procedure.

4.3.1. 1-(3-Azidophenyl)urea

NaN_3 (10 mg, 15.3 μmol) was added to a solution of 1-(3-iodophenyl)urea (20 mg, 76.6 μmol), copper(I) iodide (1.5 mg, 7.66 μmol), sodium ascorbate (3.5 mg, 17.7 μmol) and *N*,*N*′-dimethylethylenediamine (1.0 μL, 9.23 μmol) in DMSO–H_2O (5:1, 1.0 mL) at room temperature and the mixture was stirred for 10 h. The mixture was extracted with AcOEt. The organic extracts were washed with water and brine and dried over Na_2SO_4. The organic layer was evaporated and the residue was purified by flash column chromatography (toluene/acetone = 3:1) to give compound (12.6 mg, 93%) as yellow solids. mp 137–139 °C. IR (KBr) 3508, 3376, 3318, 3201, 3141, 3086, 2116, 1659, 1592, 1552, 1487, 1439, 1349, 1317, 1307 cm^{-1}. ^{1}H NMR (CD_3OD) δ 6.67 (1H, ddd, *J* = 2.0, 8.0 and 10 Hz), 7.04 (1H, ddd, *J* = 2.0, 8.5 and 10 Hz), 7.24 (1H, dd, *J* = 8.0 and 8.5 Hz), 7.31 (1H, dd, *J* = 2.0 and 2.0 Hz). ^{13}C NMR (CD_3OD) δ 110.3, 113.8, 116.4, 131.1, 141.9, 142.6, 159.1. FABMS: 178 (MH^+). Anal. Calcd for $C_7H_7N_5O$: C, 47.46; H, 3.98; N, 39.53. Found: C, 47.61; H, 4.10; N, 39.30.

4.3.2. 1-(4-Azidophenyl)urea

NaN_3 (20 mg, 30.5 μmol) was added to a solution of 1-(4-iodophenyl)urea (40 mg, 153 μmol), copper(I) iodide (2.9 mg, 15.3 μmol), sodium ascorbate (6.5 mg, 32.8 μmol) and *N*,*N*′-dimethylethylenediamine (1.7 μL, 15.6 μmol) in DMSO-H_2O (5:1, 3.0 mL) at room temperature and the mixture was stirred for 12 h. The mixture was extracted with AcOEt. The organic extracts were washed with water and brine and dried over Na_2SO_4. The organic layer was evaporated and the residue was purified by flash column chromatography (*n*-hexane/acetone = 4:1) to give compound (23.8 mg, 88%) as yellow solids. mp 174–177 °C. IR (KBr) 3418, 3351, 3318, 2575, 2491, 2449, 2141, 1613, 1581, 1534, 1512, 1485 cm^{-1}. ^{1}H NMR (CD_3OD) δ 6.96 (2H, d, *J* = 8.0 Hz), 7.38 (2H, d, *J* = 8.0 Hz). ^{13}C NMR (CD_3OD) δ 120.3, 121.9, 135.5, 138.1, 159.3. FABMS: 178 (MH^+). Anal. Calcd for $C_7H_7N_5O$: C, 47.46; H, 3.98; N, 39.53. Found: C, 47.44; H, 4.09; N, 39.13.

4.4. Click chemistry: Typical procedure

A solution of 1-(4-azidephenyl)urea (10 mM in DMSO, 3 μL) was added to a mixture of $CuSO_4$ (2 mM in H_2O, 3 μL), TBTA (2 mM in DMSO, 6 μL), sodium ascorbate (10 mM in H_2O, 3 μL), **6** (0.9 mM in H_2O, 3.3 μL) and H_2O (8.7 μL) in a 1.5-mL Eppendorf tube. The mixture was shaken at room temperature for 20 h using a shaker (1000 rpm). The whole was purified by reversed-phase HPLC [column: Waters XTerra® MS C_{18} 2.5 μm, 4.6 × 50 mm; gradient: 8–20% acetonitrile in 0.1 M triethylammonium acetate buffer (pH 7.0) for 30 min; flow rate: 1.0 mL/min] to give the desired TFO **7r**, the composition of which was confirmed by MALDI-TOF-MS analysis.

MALDI-TOF-MS data ([M−H]$^-$) for TFOs **7a–r**: **7a**, found 4528.47 (calcd 4529.06); **7b**, found 4522.96 (calcd 4521.08); **7c**, found 4573.22 (calcd 4573.16); **7d**, found 4525.32 (calcd 4525.03); **7e**, found 4523.65 (calcd 4524.04); **7f**, found 4515.98 (calcd 4515.03); **7g**, found 4530.16 (calcd 4531.03); **7h**, found 4532.02 (calcd 4532.35); **7i**, found 4559.93 (calcd 4559.04); **7j**, found 4531.39 (calcd 4531.03); **7k**, found 4544.19 (calcd 4545.06); **7l**, found 4558.90 (calcd 4559.04); **7m**, found 4573.21 (calcd 4573.07); **7n**, found 4531.03 (calcd 4529.06); **7o**, found 4527.82 (calcd 4530.05); **7p**, found 4545.18 (calcd 4545.06); **7q**, found 4559.13 (calcd 4559.04); **7r**, found 4573.53 (calcd 4573.07).

4.5. UV melting experiments (T_m measurements)

UV melting experiments were carried out on a Shimadzu UV-1650PC spectrophotometer equipped with a T_m analysis accessory. The UV melting profiles were recorded in 7 mM sodium phosphate buffer (pH 7.0), 140 mM KCl and 10 mM $MgCl_2$ or in 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM $MgCl_2$ from 5 °C to 85 °C at a scan rate of 0.5 °C/min at 260 nm. The final concentration of each oligonucleotide used was 1.5 μM or 1.9 μM. A T_m value was designated the maximum of the first derivative calculated from the profile.

Acknowledgments

This work was supported in part by Special Coordination Funds for Promoting Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT), by the Molecular Imaging Research Program from MEXT and by a Grant-in-Aid for Science Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS).

Supplementary data

Supplementary data (^{1}H and ^{13}C spectra for compound **5**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.049.

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