Pergamon

Tetrahedron Letters 43 (2002) 8755–8758

TETRAHEDRON LETTERS

# Oligonucleotides containing a nucleotide analog with an ethynylfluorobenzene as nucleobase surrogate

Niels Griesang and Clemens Richert*

*Department of Chemistry, University of Constance, Universitätsstraße 10, D-78457 Konstanz, Germany*

Received 1 August 2002; accepted 23 September 2002

**Abstract**—The 5′-protected 3′-phosphoramidite of 1-(2′-deoxy-β-D-ribofuranosyl)-2-ethynyl-4-fluorobenzene was prepared and employed in the synthesis of oligonucleotides. The duplex of CTTTTCF$^{\#}$TTCTT with AAGAAAGAAAAG, where F$^{\#}$ is the ethynylfluorobenzene-containing nucleotide, melts higher than the duplex containing a difluorotoluene moiety. 

## 1. Introduction

Nucleotide analogs with apolar aromatic rings as replacements for natural nucleobases have been shown to have interesting properties, both in terms of Watson–Crick duplexes containing these analogs and in terms of polymerase-catalyzed reactions.[1] One analog of thymidine in particular has attracted attention: the C-nucleoside containing a difluorotoluene moiety as nucleobase surrogate ('F', Fig. 1).[2] This analog is incorporated opposite to deoxyadenosine residues in polymerase-catalyzed primer extension reactions and forms base pairs that are isosteric to thymine:adenine base pairs. Therefore, this C-nucleotide may be used as a lead structure for developing new nucleotide analogs that can engage in base pairing to deoxyadenosine residues.

We have become interested in interactions between modified nucleobases and natural nucleobases that go beyond the hydrogen bonding and stacking interactions found in natural A:T and G:C base pairs. Modified nucleobases may engage in duplex stabilizing interactions by forming additional hydrogen bonds, by binding via the major or the minor groove, or by offering additional surface sites for stacking.[3,4] For example, clamp-like binding in the major groove has been reported to stabilize C:G base pairs.[5] Propinyl substituents at position 5 of pyrimidines have been shown to increase duplex stability via stacking.[6]

We have an interest in tuning the stability of T:A base pairs containing unmodified deoxyadenosine residues by modifying thymidine residues. In this case, the lack of a hydrogen bonding site at the 2-position of the adenine ring makes the formation of a third hydrogen bond difficult. Therefore, surrogates of thymine carrying substituents capable of engaging in additional van der Waals or stacking interactions, such as a fluorobenzene ring with a 2-ethynyl group (F$^{\#}$, Fig. 1), were of interest.

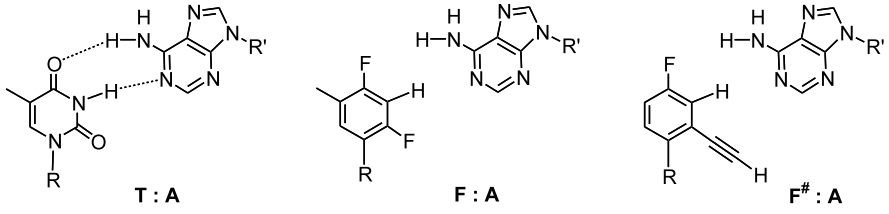



**Figure 1.**

*Keywords*: C-nucleosides; base pairing; oligonucleotides.

* Corresponding author. Present address: Institut für Organische Chemie, Universität Karlsruhe (TH), Fritz-Haber-Weg 6, D-76131 Karlsruhe, Germany. Tel. +49 (0)721 608 2091; fax: +49 (0)721 608 4825; e-mail: cr@rrg.uka.de

0040-4039/02/$ - see front matter 
PII: S0040-4039(02)02153-6

## 2. Results and discussion

Synthesis of phosphoramidite **1** (Scheme 1) started from commercially available **2**,[7] which in the present case was synthesized from less expensive 2-bromo-5-fluoroaniline in 84% yield in analogy to a protocol of Heaney and Miller for a similar substrate.[8] Sonogashira coupling[9,10] of **2** with 1.2 equivalents of trimethylsilylacetylene furnished **3** in 95% yield after distillation.[11] Bromide **3** was converted to its Grignard derivative under continuous 'starting' of the reaction with 1,2-dibromoethane, transmetallated with $CdCl_2$,[12] and reacted with chloroglycosyl donor **4**[13] to yield 53% of a mixture of epimers **5a** and **5b**.[14] The $^1H$ NMR spectrum suggested that the undesired α-anomer **5a** predominated in this mixture by a factor of 8–10. The epimers proved difficult to separate, but desilylation to **6a**/**b** in 71% yield allowed chromatographic separation, followed by epimerization of **6a** to a mixture of **6a** and **6b**.[15] The combined yield of isolated nucleosides after epimerization in toluene was 69%. Other solvents and a one pot deprotection/epimerization with $BF_3{\cdot}OEt_2$ gave lower yields. Desired β-epimer **6b**[16] was deprotected to **7** in 99% yield, 5′-protected with a dimethoxytrityl group in 79% yield, and phosphitylated under DIPAT activation[17] to obtain **1** in 76% after chromatography.

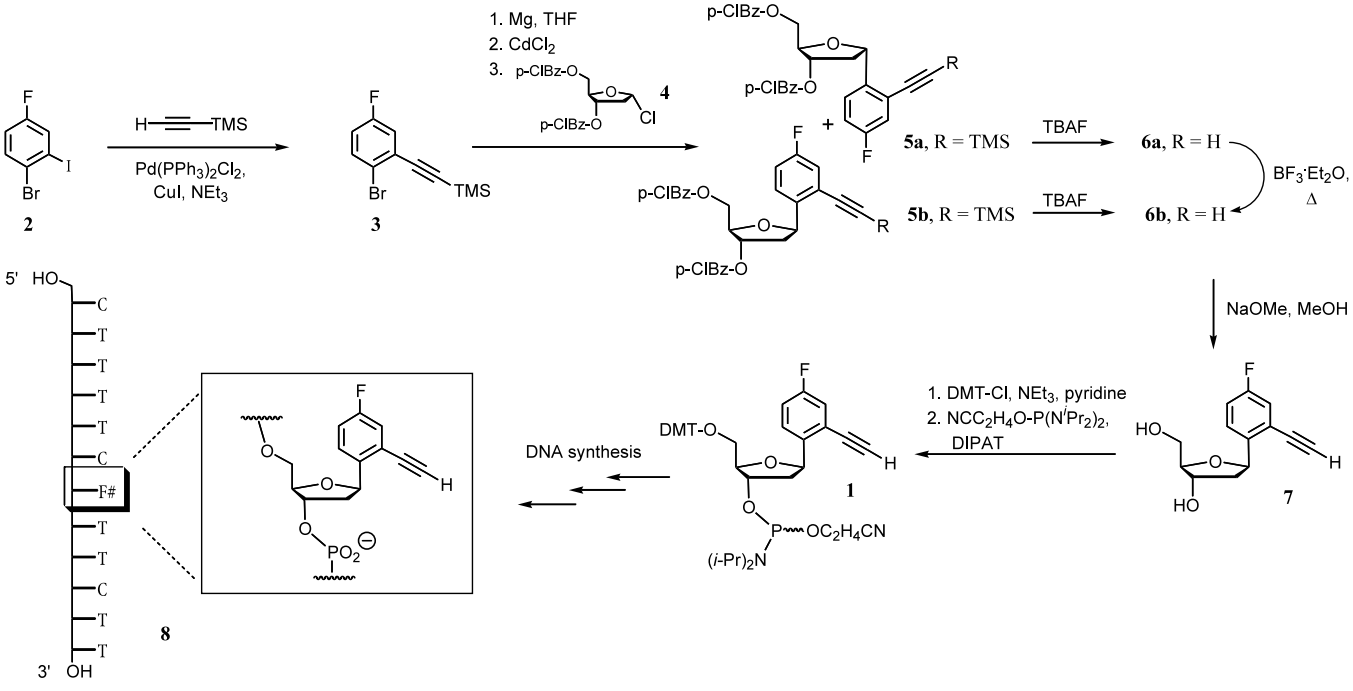
With phosphoramidite **1** in hand, DNA syntheses proceeded via a standard protocol.[18] Two modified oligonucleotides, 5′-CTTTTCF$^{\#}$TTCTT-3′ (**8**), where F$^{\#}$ denotes the nucleotide with the fluoroethynyl ring as nucleobase surrogate (Scheme 1), and 5′-TTTTAAF$^{\#}$AAT-3′ (**9**), were prepared[19] together with unmodified control and target strands. Duplexes of **9** with its complementary strand melted too low to allow determination of an accurate UV-melting point (Tm). But **8** and 5′-AAGAAAGAAAAG-3′ gave a duplex stable enough to obtain a full sigmoidal UV melting curve. Compared to unmodified duplex CTTTTCTTTCTT: AAGAAAGAAAAG, the melting point of **8**: AAGAAAGAAAAG is significantly depressed (Table 1). When compared to the duplex CTTTTCFTTCTT: AAGAAAGAAAAG, however, where a difluorotoluene moiety replaces the thymine, the duplex of **8** shows a higher melting point,[20] suggesting that the ethynyl group does provide a stabilizing effect compared to a fluoro substituent.

Given that the methyl group of difluorotoluene is absent in our base analog, the stabilizing effect of the ethynyl group may be stronger than the melting point increase suggests, since the stacking interactions pro-

**Table 1.** UV-melting points of duplexes at 1.6 μM strand concentration, 10 mM PIPES buffer, pH 7.0, 10 mM $MgCl_2$, and 100 mM NaCl, determined at 260 nm

| Duplex[a] | Tm (°C)[b] | ΔTm (°C)[c] |
|---|---|---|
| CTTTTC**T**TTCTT: AAGAAAGAAAAG | 42.2 ± 0.5 | – |
| CTTTTC**F**$^{\#}$TTCTT: AAGAAAGAAAAG | 29.5 ± 0.7 | −12.7 |
| CTTTTC**T**TTCTT: AAGAAAGAAAAG | 39.4[d] | – |
| CTTTTC**F**TTCTT: AAGAAAGAAAAG | 21.4[d] | −18.0 |

[a] Sequences are given 5′- to 3′-terminus. The residue letter at the site of modification is in boldface.
[b] UV melting point. For the first two entries, this is the mean of melting points from four curves ± one standard deviation.
[c] Melting point difference to unmodified control duplex.
[d] From Ref. 1b, see also Ref. 20.




**Scheme 1.**

vided by this methyl group are missing. Therefore it is promising to pursue ethynyl substituents at position 2 of pyrimidine nucleobase analogs further. They may also be useful for probing substrate–polymerase complexes.[21]

## Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft, grants No. RI 1063/2-1 and FOR 434, and Fonds der Chemischen Industrie. The authors wish to thank Jan Rojas, Sukunath Narayanan, and Frank Brotzel for help with DNA synthesis, UV melting curve experiments, and the acquisition of MALDI-TOF mass spectra.

## References

1. Selected references: (a) Schweitzer, B. A.; Kool, E. T. *J. Org. Chem.* **1994**, *59*, 7238–7242; (b) Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863–1872; (c) Ren, R. X.-F.; Chaudhuri, N. C.; Paris, P. L.; Rumney, S.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 7671–7678; (d) Guckian, K.; Schweitzer, B. A.; Ren, R. X.-F.; Sheils, C. J.; Paris, P. L.; Tahmassebi, D. C.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 8182–8183; (e) Moran, S.; Ren, R. X.-F.; Rumney, S.; Kool, E. T. *J. Am. Chem. Soc.* **1997**, *119*, 2056–2057; (f) Morales, J. C.; Kool, E. T. *Nature Struc. Biol.* **1998**, *5*, 950–954; (g) Matray, T. J.; Kool, E. T. *J. Am. Chem. Soc.* **1998**, *120*, 6191–6192; (h) Morales, J. C.; Kool, E. T. *J. Am. Chem. Soc.* **1999**, *121*, 2323–2324; (i) Kool, E. T. *Ann. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1–22; (j) McMinn, D. L.; Ogawa, A. K.; Wu, Y.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **1999**, *121*, 11585–11586; (k) Ogawa, A. K.; Wu, Y.; McMinn, D. L. Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 3274–3287; (l) Tae, E. J.; Wu, Y.; Xia, G.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2001**, *123*, 7439–7440; (m) Parsch, J.; Engels, J. W. *Helv. Chim. Acta* **2000**, *83*, 1791–1808; (n) Seela, F.; Bourgeois, W.; Rosemeyer, H.; Wenzel, T. *Helv. Chim. Acta* **1996**, *79*, 488–498.
2. Selected references: (a) Moran, S.; Ren, R. X.-F.; Kool, E.T. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10506–10511; (b) Guckian, K. M.; Krugh, T. R.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 6841–6847; (c) Cubero, E.; Sherer, E. C.; Luque, F. J.; Orozco, M.; Laughton, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 8653–8654.
3. For recent reviews, see e.g.: (a) Herdewijn, P. *Antisense Nucleic Acid Drug Dev.* **2000**, *10*, 297–310; (b) Kool, E. T.; Morales, J. C.; Guckian, K. M. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 990–1012, *Angew. Chem.* **2000**, *112*, 1046–1068.
4. Selected references: (a) Groebke, K.; Hunziker, J.; Fraser, W.; Peng, L.; Diederichsen, U.; Zimmermann, K.; Holzner, A. Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1998**, *81*, 375–474; (b) Fidanza, J. A.; McGall, G. H. *Nucleosides Nucleotides* **1999**, *18*, 1293–1295; (c) Nguyen, H.-K.; Fournier, O.; Asseline, U.; Dupret, D.; Thuong, N. T. *Nucleic Acids Res.* **1999**, *27*, 1492–1498; (d) Seela, F.; Becher, G. *Nucleic Acids Res.* **2001**, *29*, 2069–2078; (e) Okamoto, A.; Tanaka, K.; Saito, I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 97–99.
5. Lin, K.-Y.; Matteucci, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 8531–8532.
6. (a) Wagner, R. W.; Matteucci, M. D.; Grant, D.; Huang, T.; Froehler, B. C. *Nature Biotechn.* **1996**, *14*, 840–844; (b) Barnes, T. W.; Turner, D. H. *J. Am. Chem. Soc.* **2001**, *123*, 4107–4118 and references cited therein.
7. Available from ABCR GmbH, D-76151 Karlsruhe, Germany.
8. Heaney, H.; Millar, I. T. *Org. Syn. Collective Vol. V*, 1120–1123.
9. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *50*, 4467–4470.
10. Model protocol for a related substrate: Van Meurs, P. J.; Janssen, R. A. J. *J. Org. Chem.* **2000**, *65*, 5712–5719.
11. To a mixture of 1-bromo-4-fluoro-2-iodobenzene (**2**, 42.4 g, 0.141 mol), $Pd(PPh_3)_2Cl_2$ (990 mg, 1.41 mmol), and CuI (376 mg, 1.97 mmol) in $NEt_3$ (300 mL) under Ar was added trimethylsilylethyne (16.6 g, 23.9 mL, 0.169 mol) within 1 h. After 2 h at r.t., half conc. aqueous $NH_4Cl$ solution (400 mL) was added, the organic phase separated and the aqueous phase extracted with $CH_2Cl_2$ (3×100 mL). Distillation from the combined organic phases (0.1 Torr, 65°C) yielded **3** (36.45 g, 0.134 mol, 95%). $^1H$ NMR ($CDCl_3$, 250 MHz) $\delta$ (ppm) = 7.50 (dd, $J$ = 5.19, 8.8 Hz, 1H), 7.19 (dd, $J$ = 3.0, 8.8 Hz, 1H), 6.89 (ddd, $J$ = 3.0, 7.9, 8.8 Hz, 1H), 0.28 (s, 9H); Anal. calc: C 48.72; H 4.46, found C 48.48; H 4.42.
12. Chaudhuri, N. C.; Kool, E. T. *Tetrahedron Lett.* **1995**, *36*, 1795–1798.
13. (a) Fox, J. J.; Yung, N. C.; Wempen, I.; Hoffer, M. *J. Am. Chem. Soc.* **1961**, *83*, 4066–4068; (b) Hoffer, M. *Chem. Ber.* **1969**, *23*, 2777–2779; (c) Wang, Z. X.; Duan, W.; Wiebe, L. I.; Balzarini, J.; De Clerq, E.; Knaus, E. E. *Nucleosides Nucleotides Nucl. Acids* **2001**, *20*, 11–40.
14. Bromide **3** (16.27 g, 60 mmol) and Mg (7.3 g, 300 mmol) in THF (80 mL) were treated with 1,2-dibromoethane (11.3 g, 60 mmol) in THF (20 mL) slowly enough to maintain a mild reflux (1 h) (attention: ethene gas is liberated). The solution was heated to reflux until GC-MS showed disappearance of **3** (approx. 2 h). Solids were filtered off under Ar after cooling, and the solution treated with $CdCl_2$ (5.5 g, 30 mmol) (attention: $CdCl_2$ is very toxic and carcinogenic), followed by heating to reflux for 4 h. The mixture was cooled to 0°C and treated with **4** (11.8 g, 27.6 mmol), followed by stirring for 16 h while warming to approx. 10°C. The mixture was treated with $NH_4Cl$ solution (100 mL) and water (100 mL). The aqueous phases were extracted with $CH_2Cl_2$ (3×50 mL), the combined organic phases dried over $Na_2SO_4$, and evaporated. Flash chromatography (silica, toluene) yielded a mixture of **5a** and **5b** (8.44 g, 14.4 mmol; 53%). Rf (silica, $CH_2Cl_2$) 0.65; EI-MS (70 eV) 584, 428, 139, 111.
15. The epimers (**5a**/**b**) (560 mg, 0.956 mmol) in THF (5 mL) were treated with TBAF solution (1 M in THF, 1.15 mL) for 1 h. Sat. $NH_4Cl$ solution (15 mL) was added, followed by extraction with $CH_2Cl_2$ (3×10 mL), washing with brine, drying of the combined organic phases over $Na_2SO_4$, evaporation, and flash chromatography (silica, petroleum ether/ethyl acetate, 10:1) to yield 348 mg

(0,679 mmol, 71 %) of **6a**/**b**. Rf (silica, hexanes/ethyl acetate 4:1) 0.42 (**6a**) and 0.5 (**6b**). Epimerization of **6a** (1.1 g, 2.14 mmol) in toluene (20 mL) with $BF_3{\cdot}OEt_2$ (1.2 g, 1.08 mL, 8.56 mmol, added at rt) was induced by refluxing for 6 h. The solution was cooled, treated with $CH_2Cl_2$ (10 mL) and sat. $NaHCO_3$ solution (40 mL). The organic phase was separated, the aqueous phase was extracted with $CH_2Cl_2$ (3×20 mL), the combined organic phases concentrated and chromatographed as given above. Combined yield of **6a** and **6b**, obtained in equal amounts, 760 mg (1.48 mmol, 69%). Spectroscopic data for **6b**: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 8.01, 7.97 (2 d, $J$=8.6 Hz, 2× 2H), 7.54 (dd, $J$=8.6, 5.8 Hz, 1H), 7.47, 7.41 (2 d, $J$=8.6 Hz, 2× 2H), 7.18 (dd, $J$=8.8, 2.4 Hz, 1H), 7.03 (td, $J$=8.5, 2.8 Hz, 1H), 5.62 (dd, $J$=10.5, 5.3 Hz, 1H), 5.58 (d, $J$=6.7 Hz, 1H), 4.70 (dd, $J$=11.7, 4.2 Hz, 1H), 4.66 (dd, $J$=11.7, 4.3 Hz, 1H), 4.52 (dd, $J$=6.9, 4.2 Hz, 1H), 3.36 (s, 1H), 2.77 (dd, $J$=14.3, 4.9 Hz, 1H), 2.10–1.96 (m, 1H); $^{19}$F NMR ($CDCl_3$) $\delta$ 114.6; EI-MS (70 eV) 512, 139, 111.

16. Anomers were assigned based on the results of ROESY experiments, where a H1′ to H4′ cross peak was observed for the β-anomer, but was absent for the α-anomer, and by comparison of coupling constants and chemical shifts of non-exchangeable deoxyribose resonances with those of reference C-nucleosides known from the literature: (a) Chaudhuri, N. C.; Ren, R. X.-F.; Kool, E. T. *Synlett* **1997**, 341–347; (b) Ref. 13c.
17. Kierzek, R.; Kopp, D. W.; Edmonds, M.; Caruthers, M. H. *Nucleic Acids Res*. **1986**, *14*, 4751–4764.
18. Oligonucleotides were synthesized on an ABI 381 DNA synthesizer on a 1.0 μmolar scale, system software version 1.5. Total yields for modified oligonucleotides were within a factor of three of unmodified control strands prepared under the same conditions.
19. For **8**, HPLC: $CH_3CN$ gradient ($C_{18}$ column, 0.1 M TEAA buffer) 0% B for 5 min, to 20% B in 45 min, elution at 47 min; MALDI-TOF MS for $C_{120}H_{152}FN_{25}O_{77}P_{11}$ ([M–H]–) calc: 3537.4, found 3534.0. For **9**, HPLC: same gradient as for **8**, elution at 46 min; MALDI-TOF MS for $C_{103}H_{125}FN_{30}O_{58}P_9$ ([M–H]–) calc: 3010.1, found 3007.5.
20. Different values have been published. In Ref. 1b, the melting point is given as 21.4°C. In the erratum (*J. Am. Chem. Soc*. **1996**, *118*, 931) the authors state that α-and β-anomers give little change in results. In 1997 (Ref. 2a), the melting point is given as 27.4°C, whereas in 2000 (Ref. 3b), the authors again cite the earlier results (Ref. 1b), i.e. a melting point of 21.4°C and a ΔTm of 18.0°C. The melting point of control duplex CTTTTCTT-TCTT:AAGAAAGAAAAG at 5 μM strand concentration and identical buffer conditions is given as 39.8°C in Guckian, K. M.; Morales, J. C.; Kool, E. T. *J. Org. Chem*. **1998**, *63*, 9652–9656 and as 43.2°C in Ref. 1g. In any case, the melting point for **8** with its complement is higher than either of the melting points reported for the duplex containing difluorotoluene.
21. See e.g.: (a) Dzantiev, L.; Alekseyev, Y. O.; Morales, J. C.; Kool, E. T.; Romano, L. J. *Biochemistry* **2001**, *40*, 3215–3221; (b) Kool, E. T. *Curr. Opin. Chem. Biol*. **2000**, *4*, 602–608 and Refs 1j and 1k.