ChemComm

On-column derivatization of oligodeoxynucleotides with ferrocene†

Amy E. Beilstein and Mark W. Grinstaff*

Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708, USA. E-mail: mwg@chem.duke.edu

Received (in Bloomington, IN, USA) 16th September 1999, Accepted 21st January 2000

The solid-phase synthesis and characteristics of ferrocenoyl propargylamide (FPA)-labeled oligodeoxynucleotides are described.

Site-specifically labeled DNA is of interest for hybridization assays, artificial nucleases, anticancer therapies, and DNA-mediated charge-transfer studies.[1–16] Current oligodeoxynucleotide (ODN) labels range from metal complexes[12,17] to organic dyes[10,11,18] that are covalently attached to DNA either at the ribose,[19] phosphate[20–22] or nucleobase[4–6,23–26] moieties. While synthetic strategies such as oligodeoxynucleotide post-modification and labeled nucleoside phosphoramidites afford modified ODNs, these methods are often hampered by extensive purification, side reactions and low yields. Furthermore, many of these approaches do not allow for systematic studies to be performed, as labeling is limited to the 3′- or 5′-terminus. Here, we report the *on-column derivatization* of ODNs with ferrocene at the nucleobase using a newly developed synthetic procedure in combination with standard automated DNA synthesis.[27] This site-specific on-column procedure requires an alkyne-terminated ferrocene and a halo-substituted nucleobase for Pd(0) cross-coupling.

Ferrocene was selected as the ODN label as it possesses high stability as well as reversible and tunable electrochemical and spectroscopic properties. In addition, ferrocene has been attached previously to other biomolecules of interest, including terminal-phosphate-labeled DNA oligomers,[3,28] as well as peptide nucleic acids[29] and amino acids.[30]

The ferrocene precursor for on-column derivatization (ferrocenoyl propargylamide or FPA) (Fig. 1) was synthesized by coupling ferrocene monocarboxylic acid to propargylamine hydrochloride in the presence of dicyclohexylcarbodiimide (94% yield). To further evaluate the physical properties of these novel FPA-modified ODNs, we also synthesized the corresponding FPA-labeled nucleobase, 3′,5′-dibenzoyl-5-(ferrocenoyl propargylamide) uridine, FPAU (Fig. 1). FPAU was synthesized by Pd(0) cross-coupling[31] FPA with 3′,5′-dibenzoyloxy-5-iodouridine. FPA displayed a reversible one-electron oxidation in the cyclic voltammogram, with $E_{\frac{1}{2}}$ = 0.243 V *vs.* Ag/Ag^+ (1.0 mM compound in $CDCl_3$, 0.1 M NBu_4PF_6), compared to 0.103 V for ferrocene. After attachment to uridine, $E_{\frac{1}{2}}$ shifts to 0.262 V *vs.* Ag/Ag^+.[30] The spectroscopic properties of ferrocene, FPA and FPAU were similar: all exhibited the characteristic weak (d–d) band at 444 mm.

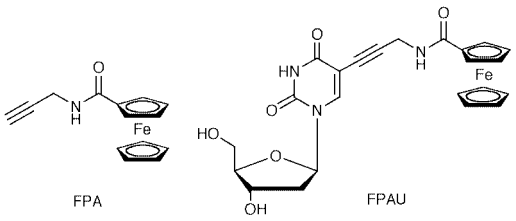
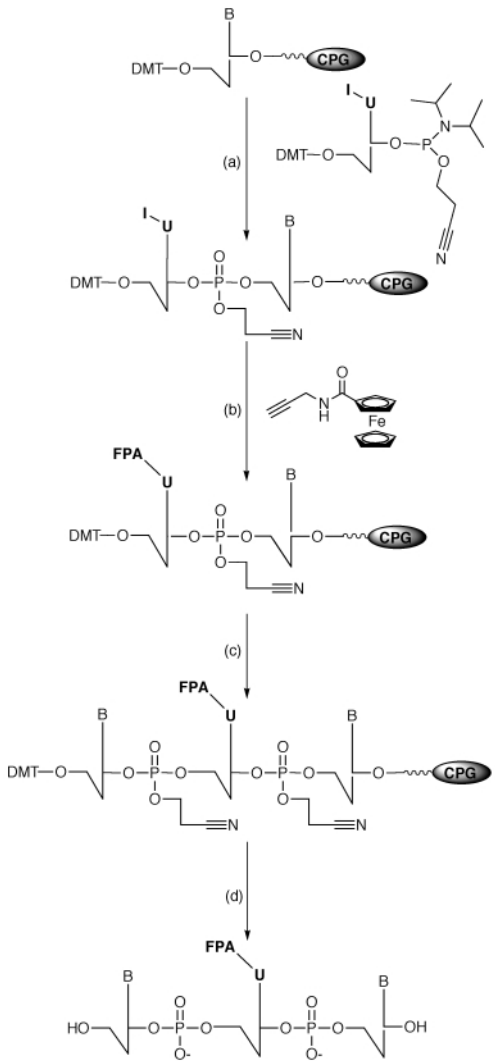


Fig. 1 Ferrocenoyl propargylamide (FPA) and 3′,5′-dibenzoyl-5-(ferrocenoyl propargylamide) uridine (FPAU).

† Electronic supplementary information (ESI) available: experimental procedures, melting profiles and CD spectra for the duplexes. See http://www.rsc.org/suppdata/cc/a9/a907568b/

The on-column derivatization method was used to site-specifically label an oligodeoxynucleotides with FPA at the nucleobase. As shown in Scheme 1, automated ODN synthesis was performed on an ABI 395 DNA synthesizer using a standard protocol. 5-Iodo-2′-deoxyuridine (5-IdU) phosphoramidite (Glen Research) was incorporated at the desired position on the ODN. Phosphoramidite couplings for the standard bases; dA, dC, dG and dT; as well as 5-IdU were > 95%. Synthesis was then paused,[32] and the column was removed from the synthesizer and dried by flushing with argon. The ferrocene precursor, FPA (20 mg), $Pd(PPh_3)_4$ (10 mg) and CuI (1 mg) were added to the column, which was then filled



Scheme 1 Key: (a) Incorporation of 5-iodo-2′-deoxyuridine phosphoramidite (5-IdU) during standard ODN synthesis (B = A, C, G or T); (b) Pd(0) cross-coupling of FPA and the column-bound iodouridine; (c) normal ODN synthesis is resumed; (d) the synthesized site-specifically FPA-labeled ODN is cleaved from the column and protecting groups are removed by incubation in ammonia at 55 °C for 16 h.

DOI: 10.1039/a907568b

Table 1 Melting temperatures for FPA-labeled and unlabeled duplexes

Duplex		T_m/°C
1	5′-TGC TAC AAA CTG T**U**^{FPA}G A-3′	
4	5′-TCA ACA GTT TGT AGC A-3′	49.6 ± 0.1
2	5′-TGC TAC AAA C**U**^{FPA}G TTG A-3′	
4	5′-TCA ACA GTT TGT AGC A-3′	48.2 ± 0.2
3	5′-TGC TAC AAA CTG TTG A-3′	
4	5′-TCA ACA GTT TGT AGC A-3′	50.8 ± 0.3
5	5′-TGC TAC AAA C**U**^{FPA}G-3′	
7	5′-CAG TTT GTA GCA-3′	42.6 ± 0.3
6	5′-TGC TAC AAA CTG-3′	
7	5′-CAG TTT GTA GCA-3′	41.4 ± 0.4

(**U**^{FPA}) indicates the FPA-labeled uridine

with DMF–TEA (9:1). The column was shaken at room temperature for 6 h. It was then rinsed with DMF–TEA, dried and replaced on the synthesizer. Routine synthesis was resumed until the desired oligodeoxynucleotide was synthesized. A series of FPA-labeled ODNs were synthesized in this manner (Table 1). FPA-modified oligodeoxynucleotides were purified *via* reverse-phase HPLC; retention times for labeled and unlabeled ODNs differed by several minutes (18.8 *cf.* 14.8 min). Isolated yields of the labeled ODNs were typically 50%.[33]

The stabilities of FPA-labeled duplexes are evaluated by thermal denaturation of the duplexes.[34] Table 1 lists the melting temperatures of several FPA-labeled and unmodified duplexes. FPA-modified duplex **1**:**4** which is labeled at the third base from the 3′-terminus, exhibits similar stability to duplex **2**:**4**, labeled at the sixth base. These melting temperatures are only slightly lower than the T_m for the unmodified duplex, **3**:**4**. Duplex **5**:**7**, a dodecamer labeled at the second base, has a T_m of 42.6 *cf.* 41.4 °C for the analogous unmodified duplex **6**:**7**. The small changes in the melting temperatures of the labeled duplexes compared with the unmodified duplexes indicate that FPA derivatization causes minimal disruption of duplex structure. These data are consistent with previous reports that the 5-position of uridine tolerates a variety of modifications without disrupting the duplex.[26] CD spectra of the duplexes also indicate that the FPA-labeled duplexes retain the B-form DNA structure found in the unlabeled duplexes.

In summary, novel ODNs labeled with ferrocene are synthesized by solid-phase coupling of a ferrocene derivative to an ODN containing 5-iodouridine. This facile synthesis provides a simple method for labeling ODNs at the nucleobase with a spectroscopic or redox label in good yields, with minimal purification of the labeled ODN product. Melting profiles and CD spectra (see ESI) show that the FPA-labeled duplexes are stable at room temperature and have B-form DNA structure similar to unlabeled duplexes.

This work was supported in part by NSF (CAREER), Army Research Office, and Duke University.

Notes and references

1 U. Diederichsen, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2317.
2 M. W. Grinstaff, *Angew. Chem., Int. Ed.*, 1999, **38**, 3629 and references therein.
3 T. Ihara, M. Nakayama, M. Murata, K. Nakano and M. Maeda, *Chem. Commun.*, 1997, 1609.
4 G. B. Dreyer and P. B. Dervan, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 968.
5 R. Manchanda, S. U. Dunham and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 5144.
6 J. Schliepe, U. Berghoff, B. Lippert and D. Cech, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 646.
7 R. E. Holmlin, P. J. Dandliker and J. K. Barton, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2714 and references therein.
8 A. M. Brun and A. Harriman, *J. Am. Chem. Soc.*, 1994, **116**, 10 383.
9 T. J. Meade and J. F. Kayyem, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 352.
10 F. D. Lewis, R. Wu, Y. Zhang, R. L. Letsinger, S. R. Greenfield and M. R. Wasielewski, *Science*, 1997, **277**, 673.
11 S. M. Gasper and G. B. Schuster, *J. Am. Chem. Soc.*, 1997, **119**, 12762.
12 P. G. Sammes and G. Yahioglu, *Nat. Prod. Rep.*, 1996, 1.
13 A. Sigel and H. Sigel, *Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules; Metal Ions in Biological Systems*, Marcel Dekker Inc., New York, 1996, vol. 33.
14 D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180.
15 T. L. Netzel, *Organic and Inorganic Photochemistry*, Marcel Dekker, New York, 1998.
16 M. J. Clarke, *Adv. Chem. Ser.*, 1997, **253**, 349.
17 R. E. Holmlin, R. T. Tong and J. K. Barton, *J. Am. Chem. Soc.*, 1998, **120**, 9724.
18 K. Fukui and K. Tanaka, *Angew. Chem., Int. Ed.*, 1998, **37**, 158.
19 E. Meggers, D. Kusch, M. Spichty, U. Wille and B. Giese, *Angew. Chem., Int. Ed.*, 1998, **37**, 460.
20 W. Bannwarth and D. Schmidt, *Tetrahedron Lett.*, 1989, **30**, 1513.
21 R. E. Holmlin, P. J. Dandliker and J. K. Barton, *Bioconjugate Chem.*, 1999, **10**, 1122.
22 S. I. Khan, A. E. Beilstein, M. Sykora, G. D. Smith, X. Hu and M. W. Grinstaff, *Inorg. Chem.*, 1999, **38**, 3922.
23 J. Telser, K. A. Cruickshank, K. S. Schanze and T. L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 7221.
24 D. J. Hurley and Y. Tor, *J. Am. Chem. Soc.*, 1998, **120**, 2194.
25 S. I. Khan, A. E. Beilstein and M. W. Grinstaff, *Inorg. Chem.*, 1999, **38**, 418.
26 J. D. Kahl and M. M. Greenberg, *J. Am. Chem. Soc.*, 1999, **121**, 597.
27 S. I. Khan and M. W. Grinstaff, *J. Am. Chem. Soc.*, 1999, **121**, 4704.
28 R. C. Mucic, M. K. Herrlein, C. A. Mirkin and R. L. Letsinger, *Chem. Commun.*, 1996, 555.
29 A. Hess and N. Metzler-Nolte, *Chem. Commun.*, 1999, 885.
30 H. B. Kraatz, J. Lutsztyk and G. D. Enright, *Inorg. Chem.*, 1997, **36**, 2400.
31 K. Sonogashira, Y. Tohda and N. Hagihara, *Tetrahedron Lett.*, 1975, **50**, 4467.
32 Interruption of DNA synthesis to perform the coupling reaction resulted in higher yields than synthesizing the oligonucleotide in entirety before coupling to FPA (50 *cf.* 8% yield).
33 The labeled ODNs were confirmed by ESI-MS in the negative mode (**1**: (M − 2)/2, (M − 3)/3 and (M − 4)/4 ions found, with a reconstructed mass of 5132.84, compared to the calculated molecular mass of 5131.187).
34 Equimolar amounts of complimentary single strands were combined to give a duplex solution of absorbance of *ca.* 0.5. The mixture was heated to 80 °C for 15 min and was then cooled to ambient temperature over 3 h. After cooling, the following parameters were used for the melting experiment: (a) monitoring wavelength, 260 nm; (b) temperature range, 20–70 °C; (c) temperature step, 0.5 °C; (d) integration time 1 s; and wait time 1.0 min. Melting temperatures were determined by the first derivative of the melting profile and were repeated multiply for each duplex.

Communication a907568b