Supplementary Material (ESI) for Chemical Communications

In-situ azide formation and "click" reaction of nile red with DNA as an alternative postsynthetic route

Christoph Beyer and Hans-Achim Wagenknecht*

Supporting Information

University of Regensburg
Institute for Organic Chemistry
93040 Regensburg, Germany
Fax +49 941 943 4802
E-mail: achim.wagenknecht@chemie.uni-regensburg,de

Experimentals

General. Unless otherwise specified, commercial reagents and starting materials were purchased from commercial suppliers and used without further purification. Purified water with a resistivity ≥ 18 MΩ cm^{-1} was used for preparation of buffer solvents. 5'-Dimethoxytrityl-5-iodo-2'-deoxyUridine,3'-[(2-cyanoethyl)(*N*,*N*-diisopropyl)]-phosphoramidite was purchased from Glen Research. Unmodified oligonucleotides were purchased from Metabion. nile red[1] and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine[2] were synthesized according to literature synthesis procedures.Unless otherwise stated, all reactions were carried out at r.t.. Synthetic steps involving azides were performed in the dark. Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry on a 1 μmol scale. Reagents and controlled pore glass (CPG) were purchased from ABI and Glen Research. 5-iodo-modified uridine was introduced into DNA by using standard coupling conditions.

Synthesis of oligodeoxynucleotides and on-bead coupling . Iodo-modified DNA on CPG was dried under high vacuum after DNA synthesis. 1.0 mL sodium azide (200 mM) solution in DMSO was added. The reaction mixture was heated at 55°C for 1 h and cooled to r.t. The vial was centrifuged and the supernatant was removed. The CPG was successively washed with DMSO (4 mL) and MeCN (4 mL). Nile Red (300 μL, 50 mM), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (600 μL, 100 mM), tetrakis(acetonitrile)copper(I)-hexafluorophosphate (300 μL, 100 mM) (each in DMSO/^{t}BuOH 3:1) and (+)-sodium L-ascorbate (300 μL, 400 mM in H_2O) was added to the CPG and the mixture was gently shaken overnight at r.t. The suspension was centrifuged and the supernatant removed. The CPG was successively washed with DMSO, EtOH and H_2O (each 2 mL) and dried under high vacuum. The oligomers were deprotected by concentrated NH_4OH solution at r.t. for 18 h.

DNA purification. After deprotection from CPG the DNA was desalted by NAP-5 column (GE Healthcare). The modified oligonucleotide was purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A) NH_4OAc buffer (50 mM); B) acetonitrile; gradient 0-30% B over 45 min, flow rate 2.5 mL/min, UV/Vis detection at 260 and 600 nm. Mass spectra of the purified oligonucleotides were recorded at the University of Regensburg, Zentrale Analytik Massenspektrometrie, with a ThermoQuest Finnigan TSQ 7000 in negative and positive ionization mode on a Discovery Bio Wide C18 3μ 100 x 2.1 mm column using the following conditions: A) NH_4OAc buffer (10 mM); B)

acetonitrile; gradient 0-90 % B over 11 min, then 90% B over 2 min, then 90-0 % B over 1 min, then 0 % B over 5 min, with a flow rate of 0.3 mL/min, UV/Vis detection. The purified oligonucleotide was identified by ESI-MS m/z: calcd: 5554, found: 1386.6 $[M-4H]^{4-}$,1849.4 $[M-3H]^{3-}$, 1388.6 $[M+4H]^{4+,}$1851.6 $[M+3H]^{3+}$. The oligonucleotide was quantified by its absorbance at 260 nm using ε_{260nm}= 28,000 M^{-1} $^{cm-1}$ for nile red-dU.

Optical spectroscopy. The oligonucleotides were lyophilized and quantified by their absorbance in 10 mM sodium phosphate buffer at 260 nm on a Varian Cary 100 spectrometer. Duplexes were formed by heating to 90 °C (10 min) followed by slow cooling. Absorption spectra and melting temperature (2.5 μM DNA, 20-90 °C, 0.7 °C/min, step width 0.5 °C) were recorded on a Varian Cary 100 spectrometer equipped with a 6×6 cell changer unit. Fluorescence spectra were measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a stepwidth of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 5 nm and are corrected for Raman emission from the buffer solution. The fluorescence quantum yields (Φ_{Fl}) were determined by the standard method, taking into account the refractive indices (n) of the solvents (eq S1):

$$\phi_{Fl} = \phi_R \frac{A_R}{A_S} \frac{F_S}{F_R} \frac{n_S^2}{n_R^2} \qquad \text{(Eq. S1)}$$

The subscripts "*S*" and "*R*" refer to the sample and the reference dye, respectively. *A* is the extinction of the sample solution at the excitation wavelength; *F* is the emission integral over the area of interest. The fluorescence quantum yields were determined by the standard method with Cresyl Violet perchlorate in MeOH as reference (Φ_{Fl} = 0.54).[3]

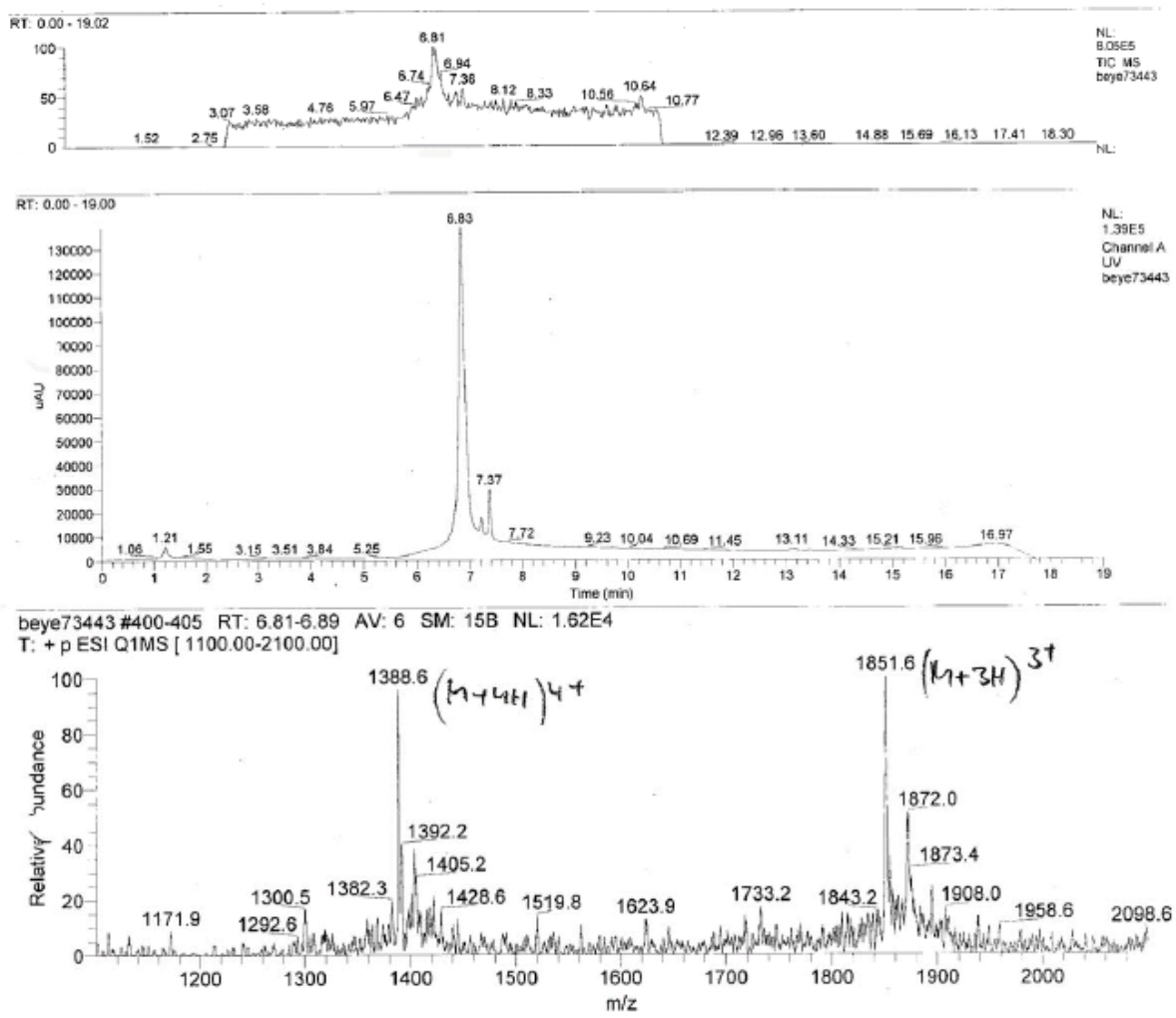

Figure S1. Analytical LC-MS of **DNA1**, positive ionization mode

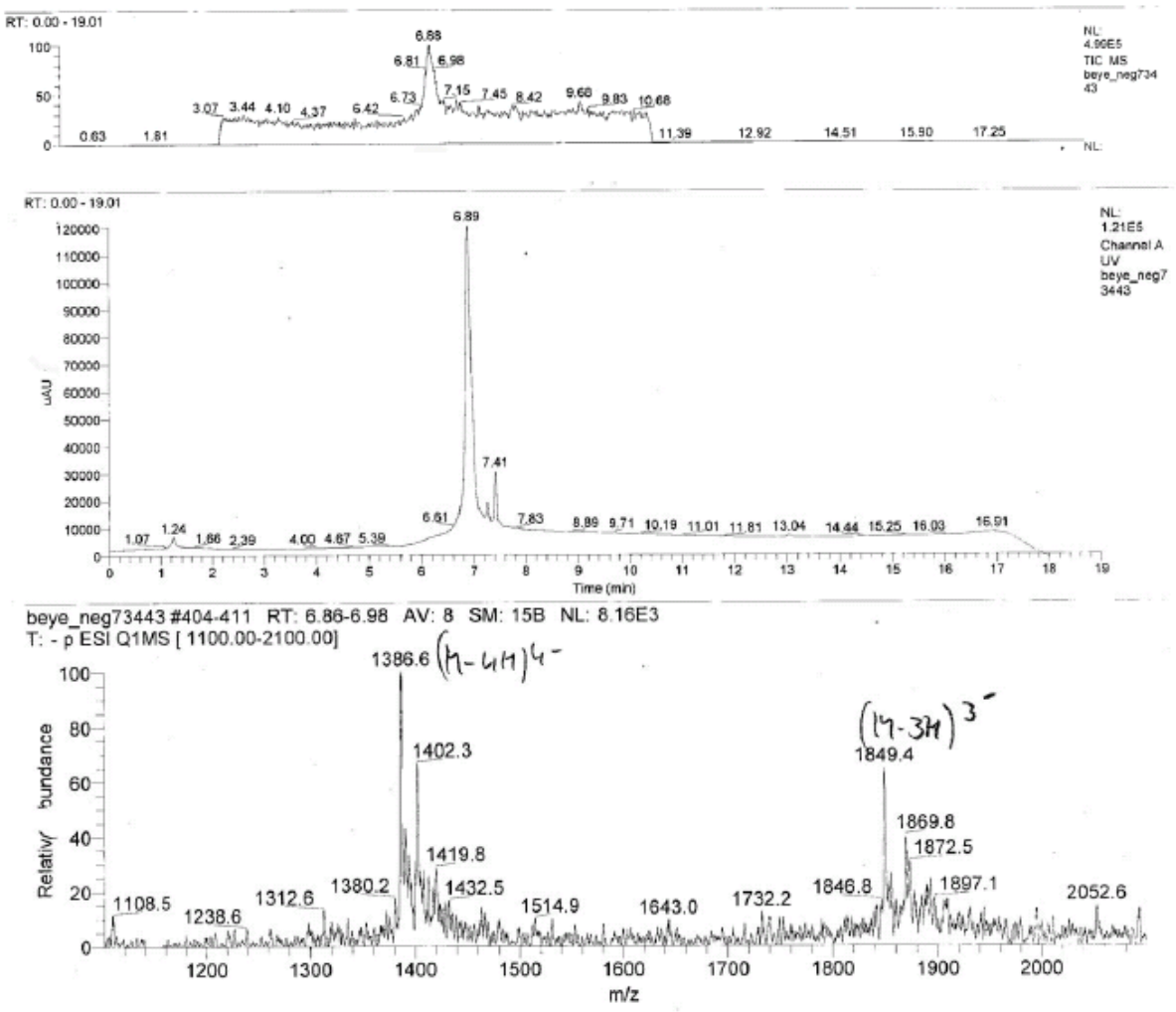

Figure S2. Analytical LC-MS of **DNA1**, negative ionization mode

[1] J. Han, J. Jose, E. Mei, K. Burgess, *Angew. Chem., Int. Ed.* **2007**, *46*, 1684.
[2] B.-Y. Lee, S. R. Park, H. B. Jeon, K. S. Kim, *Tetrahedron Lett.* **2006**, *47*, 5105.
[3] M. L. Deda, M. Ghedini, I. Aiello, T. Pugliese, F. Barigelletti, G. Accorsi, *J. Organomet. Chem.* **2005**, *690*, 857.