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ORIGINAL PAPER

Selectivity of a bromoacridine-containing fluorophore for triplex DNA

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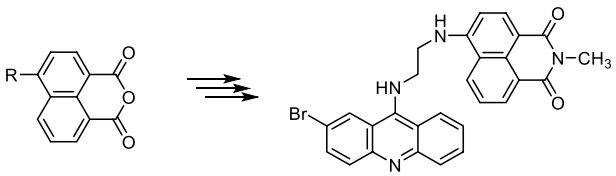
Abstract
Fluorophore 1,8-naphthilamide was linked to 2-bromoacridine through an ethylenediamine spacer using a succinct synthetic route to give a bromoacridine-linked, bifunctional fluorophore conjugate for the detection of triplex DNA. Acridine is well known to intercalate into duplex DNA whereas introduction of a bulky bromine atom at position C2 redirects specificity for triplex over duplex DNA. In this work, photoelectron transfer assay was used to demonstrate that the synthesised 2-bromoacridine-linked fluorophore conjugate had good selectivity for the representative triplex DNA target sequence $d(T*A.T)_{20}$ compared with double-stranded $d(T.A)_{20}$, single-stranded dT_{20} or $d(G/A)_{19}$ DNA sequences.

Graphic abstract



Keywords Nucleic acids · Bisensors · Fluorescence spectroscopy · Heterocycles

Introduction

The use of triplex-forming oligonucleotides allows potentially sequence-specific targeting of genes in humans and in other organisms to control or alter gene expression [1]. The structural and assembly characteristics that typify DNA triplexes allow the design of DNA nanonstructures for structural applications and targeting of non-nucleic acid components [2]. Representative triplex structures are shown, whereby the Watson–Crick A.T and G.C DNA base pairs are targeted by T (Fig. 1, left) and protonated C^+ (Fig. 1, right) [3].

Of the various methods that exist for the study of DNA triplexes, variable temperature UV analysis and non-denaturing polyacrylamide gel electrophoresis (PAGE) are commonly used. There are examples where stable DNA triplexes are expected to form but fail to show temperature-dependant changes in hyperchromicity thus rendering triplex formation "invisible" to UV analysis in such instances [4]. The use of non-denaturing PAGE is highly effective for both the qualitative and quantitative analyses of DNA triplexes but the use of radioactive material containing ^{32}P and/or other radionuclides requires expertise in handling, manufacture and the safe disposal of waste to protect against negative impact on the environment. Fluorescence-based [5] approaches provide potentially less expensive, greener methods for triplex DNA analysis. The use of a bifunctional triplex DNA sensor incorporating the 1,8-naphthilamide fluorophore and a naphthoquinoline motif was examined [6] and shown to be selective for triplex over duplex and single-stranded DNA, as judged by photoelectron transfer [7] (PET) assay.

We wished to examine whether the PET assay approach could be extended to the use of the acridine conjugate **7** with the simplicity of using bromine substitution within the acridine ligand to promote recognition and binding to triplex DNA. Normally, acridine acts as a potent duplex DNA intercalator [8, 9] but the presence of bromine atom at position

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C2 has been shown to redirect its specificity for triplex DNA [10].

Results and discussion

To synthesise conjugate **7**, we first prepared 2-bromo-9-chloroacridine (**3**) (Scheme 1). *N*-(4-Bromophenyl)anthranilic acid (**2**) was prepared from 2-chlorobenzoic acid (**1**) by a literature modification to the Ullman synthesis [11]. The anthranilic acid **2** was then cyclised with concomitant chlorination to give the acridine **3** [12, 13].

The preparation of the 4-bromonaphthalimide **6** was achieved by reacting compound **4** with methylamine [14] to give compound **5**, that was subsequently treated with ethylenediamine [15] (Scheme 2). The coupling of acridine **3** to naphthalimide **6** was successful and gave the target conjugate **7**, albeit it in very low yield. The structures of

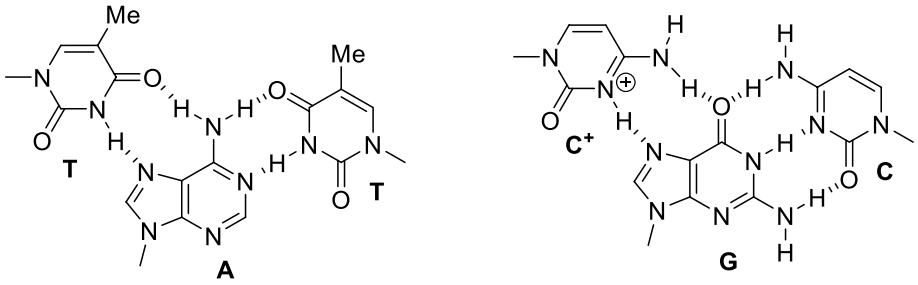
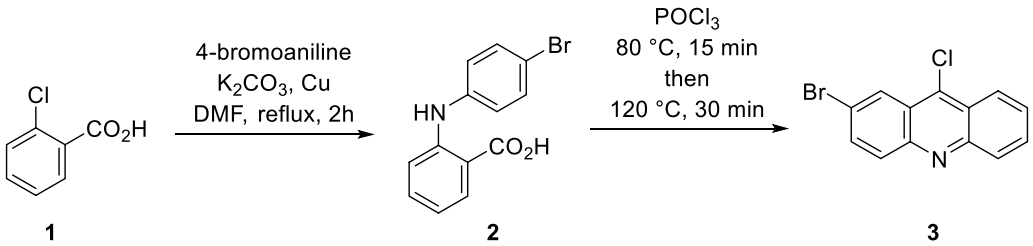
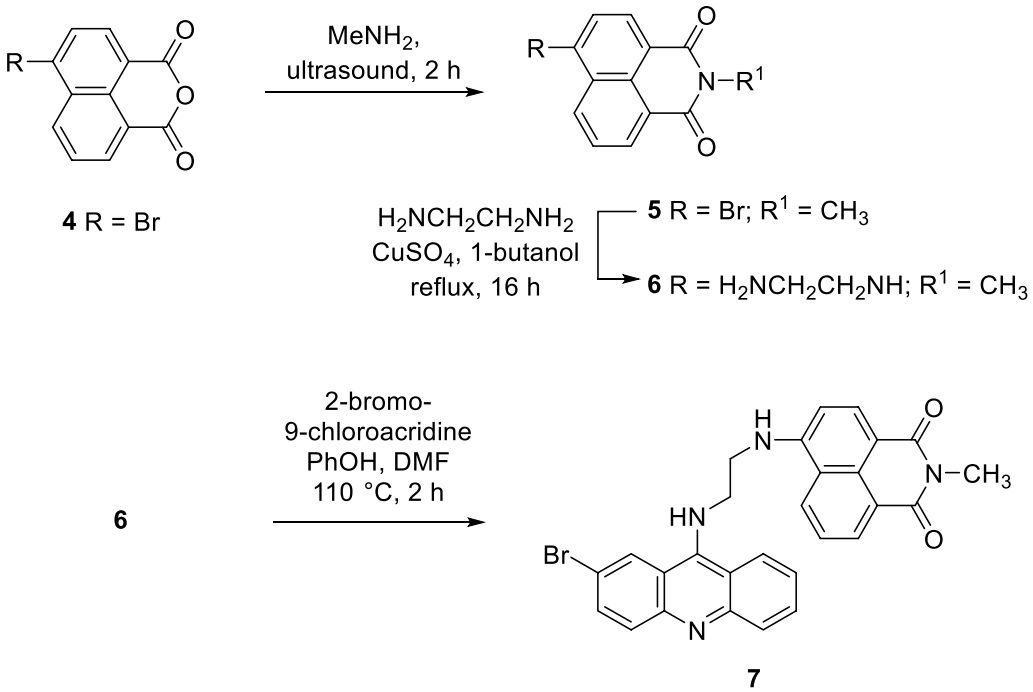


Fig. 1 Representative DNA triplex structures T*A.T (left) and C+*G.C (right)

Scheme 1



Scheme 2



conjugate **7** and the intermediates were consistent with their spectroscopic properties. Representative spectroscopic data are given in the Supplementary Material.

DNA sequences dT_{20}, dA_{20} and the mixed G/A sequence were prepared by solid phase synthesis using phosphoramidites on a Beckman Oligo 1000 DNA synthesiser according to the manufacturer's protocol. The oligonucleotide products were purified by reversed phase HPLC. Representative HPLC chromatograms are given in the Supplementary Material.

The increase in fluorescence enhancement shown by conjugate **7** in the presence of triplex DNA, correlated with the increase in concentration of the DNA triplex, as evidenced by the PET assay results summarised in Fig. 2. To examine the selectivity for triplex DNA, conjugate **7** (1 μM) was separately added to the same total concentration (40 μM) of triplex (TAT), duplex (AT), two types of single-stranded DNA (T and G/A), and the fluorescence enhancements were examined by PET assay (Fig. 3). Conjugate **7** was not specific for triplex DNA but it did, however, show marked selectivity for triplex DNA compared with the duplex DNA and single-stranded DNA sequences.

Conclusion

We have shown conjugate **7** to be selective for triplex DNA compared with duplex or single-stranded DNA. Although its discriminatory properties were not superior to the naphthoquinoline conjugate described by Lu and co-workers [6], its

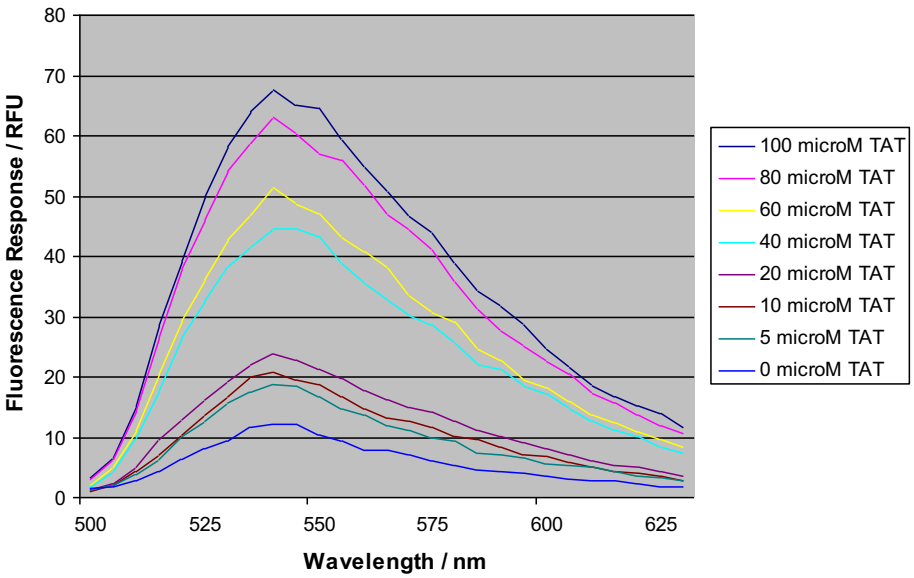


Fig. 2 Emission spectra (excitation at 457 nm) of conjugate **7** (1 μM) in the presence of various concentrations of DNA triplex $d(T^*A.T)_{20}$ ranging from 0, 5, 10, 20, 40, 60, 80, and 100 μM measured in 25 mM Tris.HCl buffer containing 10 mM $MgCl_2$, 100 mM NaCl, and 0.5 mM spermine at pH 7. Fluorescence is given in relative fluorescence units (RFU)

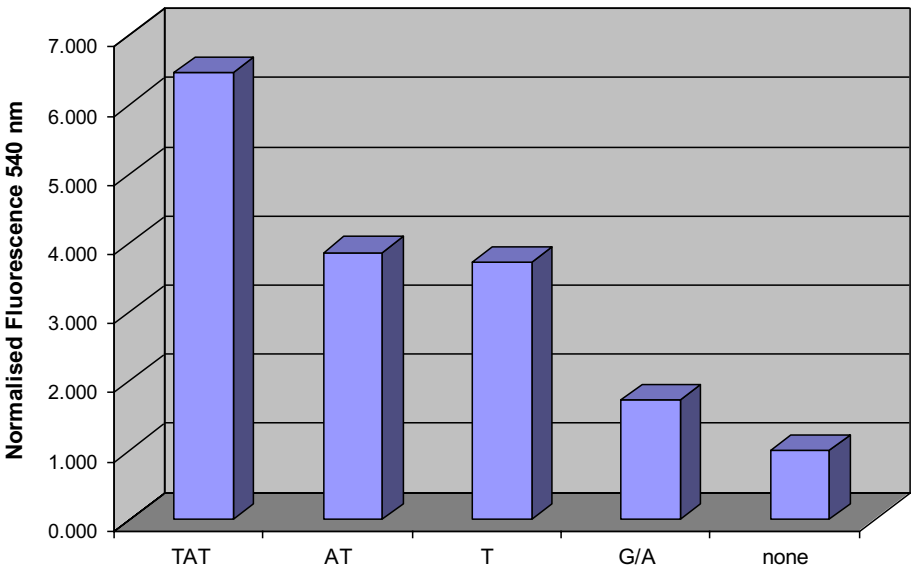


Fig. 3 Fluorescence response of conjugate **7** (1 μM) to various nucleic acid samples (40 μM) in 25 mM Tris.HCl buffer containing 10 mM $MgCl_2$, 100 mM NaCl, and 0.5 mM spermine at pH 7. TAT represents the triplex formed from 2 molar equivalents of dT_{20} and 1 molar equivalent of dA_{20}; AT represents the duplex formed from 1 molar equivalent of dT_{20} and 1 molar equivalent of dA_{20}; T represents single-stranded dT_{20}; G/A represents the 19-mer sequence 5′-dAGAGAGGAG AGAAGAGGAG; none represents the fluorescence response in the absence of nucleic acid

concise synthesis should allow access to further substitution patterns in the acridine portion to potentially fine-tune the selectivity for triplex DNA.

Experimental

NMR spectra were recorded on a Bruker AC-250 spectrometer in $CDCl_3$ or DMSO-d_6. The chemical shift values are expressed as δ values (ppm) down field with residual protons of the solvents ($CDCl_3$, $\delta = 7.26$ ppm; DMSO-d_6, $\delta = 2.49$ ppm) as internal standards. Mass spectrometric analyses were performed in FAB + mode using a VG AutoSpec instrument. Infrared spectra were recorded using a Mattson Galaxy 2020 FT-IR Spectrophotometer. Melting points were measured on a Gallenkamp Electrothermal Digital apparatus. Fluorescence spectra were recorded on a Spectramax Gemini XS dual-scanning microplate spectrofluorometer. Oligonucleotides were prepared on a Beckman Oligo 1000 DNA synthesiser following the manufacturer's protocol using commercially available reagents (LINK Technologies). Purification of oligonucleotides was performed by semi-preparative reversed phase HPLC. The procedures for preparation of known compounds **2** [11], **3** [12, 13], **5** [14], **6** [15] and their analytical and spectroscopic data are given in the Supplementary Material.

6-[2-[(2-Bromoacridin-9-yl)amino]-ethylamino]-2-methylbenzo[de]isoquinoline-1,3-dione (7, $C_{28}H_{21}BrN_4O_2$) To an oven dried two-necked round bottom flask (100 cm^3) was added 153 mg 2-bromo-9-chloroacridine (**3**, 0.52 mmol) and 983 mg phenol (10.46 mmol) under an argon atmosphere and the flask heated (80 °C) until the acridine had dissolved. The 1,8-naphthalimide **6** (128 mg, 0.48 mmol) was dissolved in the minimum of dry DMF, added to the reaction flask and the reaction mixture heated (110 °C) with stirring (2 h). The product mixture was then allowed to cool (rt) before addition of chilled ethanol (3 cm^3). After refrigeration (overnight), the precipitate was collected by Buchner filtration and dried under vacuum to give the product (37 mg, 13%) as an orange solid. TLC: $R_f = 0.78$ (acetone-MeOH 20:1); IR: $\overline{V} = 3379$, 3246, 3067, 2934, 1678, 1638, 1572, 1475, 1359, 1277, 1124, 756 cm^{-1}; ^1H NMR: $\delta = 9.37$ (1H, s, NH), 8.75 (1H, br s, NH), 8.43–8.31 (2H, m, 2×CH), 8.05 (1H, d, $J = 8.8$ Hz, CH), 7.89–7.75 (2H, m, 2×CH), 7.49–7.35 (2H, m, 2×CH), 7.15 (2H, t, $J = 7.5$ Hz, 2×CH), 6.81–6.72 (3H, m, 3×CH), 4.50 (2H, br s, CH_2), 3.94 (2H, br s, CH_2), 3.39 (3H, s, CH_3) ppm; ^{13}C NMR: $\delta = 158.0$, 150.1, 141.5, 137.6, 128.5, 125.9, 124.2, 123.2, 121.2, 121.1, 120.3, 118.4, 108.2, 104.2, 47.3, 42.5, 26.3 ppm; MS (APCI$^-$): $m/z = 523$, 525; calcd for $C_{28}H_{21}BrN_4O_2$ ([M-H]$^-$) 523.0770, found 523.0792.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00706-021-02816-5.

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