Bioorganic & Medicinal Chemistry 25 (2017) 3574–3582

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design and synthesis of novel photoinduced electron transfer-based hybridization probes

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ARTICLE INFO

Article history:
Received 12 April 2017
Revised 4 May 2017
Accepted 5 May 2017
Available online 6 May 2017

Keywords:
Oligonucleotide
DNA
RNA
Pet
Fluorescence
Detection

ABSTRACT

Photoinduced electron transfer (PeT)-based hybridization probe is a linear and quencher-free oligonucleotide (ON) probe for DNA or RNA detection. In this report, we designed and synthesized novel adenosine analogues for PeT-based hybridization probe. In particular, the analogue containing a piperazinomethyl moiety showed effective quenching property under physiological conditions. When the probe containing the analogue was hybridized with a complementary DNA or RNA, the fluorescence increased 3- or 4-fold, respectively, compared to the single-stranded state.

1. Introduction

Oligonucleotide (ON) hybridization probes are widely used in the field of molecular biology.[1] Discovery of a large number of non-coding RNAs (ncRNAs)[2] have resulted in increasing reports of the synthesis of such chemically modified ON probes in recent years.[3–14] Molecular beacon (MB) is a single-stranded ON probe with a stem-loop hairpin shaped structure.[3–5] In the stem-loop structure, the fluorophore in the MB is close to the quencher, resulting in quenching of the fluorescence. Hybridization with a target strand results in conformational change of MB, and fluorescence emission is observed. The conformational change often results in slow response of MB. Furthermore, residual quenching may limit MB brightness. Therefore, several linear or quencher-free ON probes have been developed.[6–14]

Recently, we reported the synthesis of photoinduced electron transfer (PeT) based quencher-free hybridization probe (Fig. 1).[15] Fluorescence of anthracene moiety is quenched by electron flow from the adjacent amine to the empty highest occupied molecular orbital (HOMO). Hybridization of ON probe containing **1** with complementary DNA or RNA results in protonation of the amino residue, **1**, from phosphate of the complementary strand. This protonation inhibits PeT from the amino residue, resulting in fluorescence emission. We successfully detected complementary DNA and RNA strands by using **1** containing linear ON probe. However, the quenching of ON probes containing analogue **1** was thought to be insufficient under physiological conditions because the pK_a value of the amino moiety of **1** was 7.94.[15]

To overcome this problem, we have designed novel adenosine analogues **2**–**4** (Fig. 2). The pK_a values of piperazine (9.8), morpholine (8.5), which are the amino moieties of **2** and **3**, are lower than that of dimethyl amine (10.7).[16] The pK_a value of bis(2-methoxyethyl)amine, which is the amino moiety of **4**, is also expected to be lower than that of dimethyl amine because of the electronegative substituents. Thus, we expected that ON probes containing **2**–**4** would show effective fluorescence quenching, even under physiological conditions.

In this work, we first synthesized the adenosine analogues **2**–**4** and evaluated their fluorescent properties. Only analogue **2** showed PeT based fluorescence quenching ability, and its pK_a value was calculated as 3.13. Therefore, we next synthesized ON probes containing analogue **2** for detection of complementary RNA and DNA strands.

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http://dx.doi.org/10.1016/j.bmc.2017.05.013
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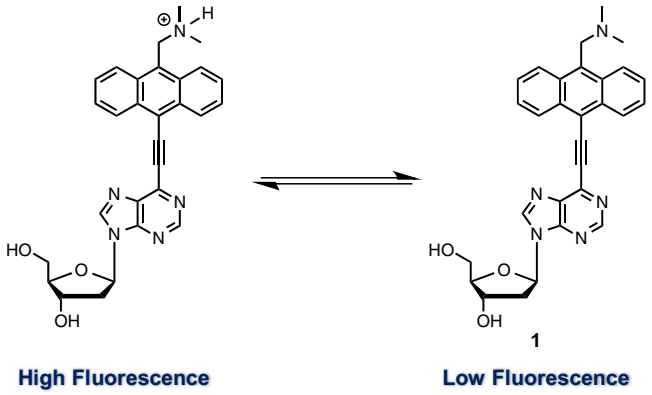


Fig. 1. The PeT-based fluorescence quenching mechanism of nucleoside analogue **1**.

2. Results and discussion

2.1. Synthesis of nucleoside analogues

Methods for synthesis of nucleoside analogues **2**–**4** are shown in Schemes 1–3. First, we synthesized fluorescence moiety of the nucleoside analogue from 10-chloro-9-anthraldehyde (**6**). 10-chloro-9-morpholinoanthracene (**9**) was synthesized by reductive amination reaction of **6** with morpholine (**7**) in the presence of 2-picoline borane. Compound **9** was coupled with triisopropylsilylacetylene (TIPS-acetylene) in the presence of $PdCl_2(CH_3CN)_2$, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-Phos) and Cs_2CO_3 in MeOH at 90 °C to produce the ethynyl derivative **11** with a yield of 64%. The TIPS group was deprotected using tetra-*n*-butylammonium fluoride (TBAF) to afford 80% yield of the anthracene derivative **13**. Compound **14** was also synthesized from **6** and bis

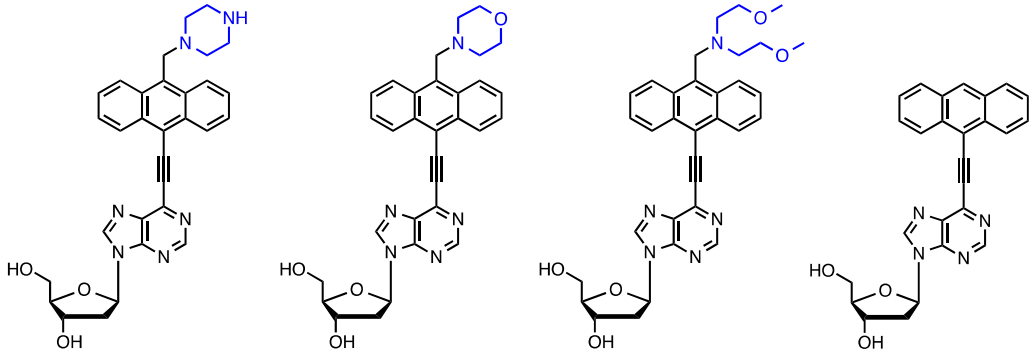
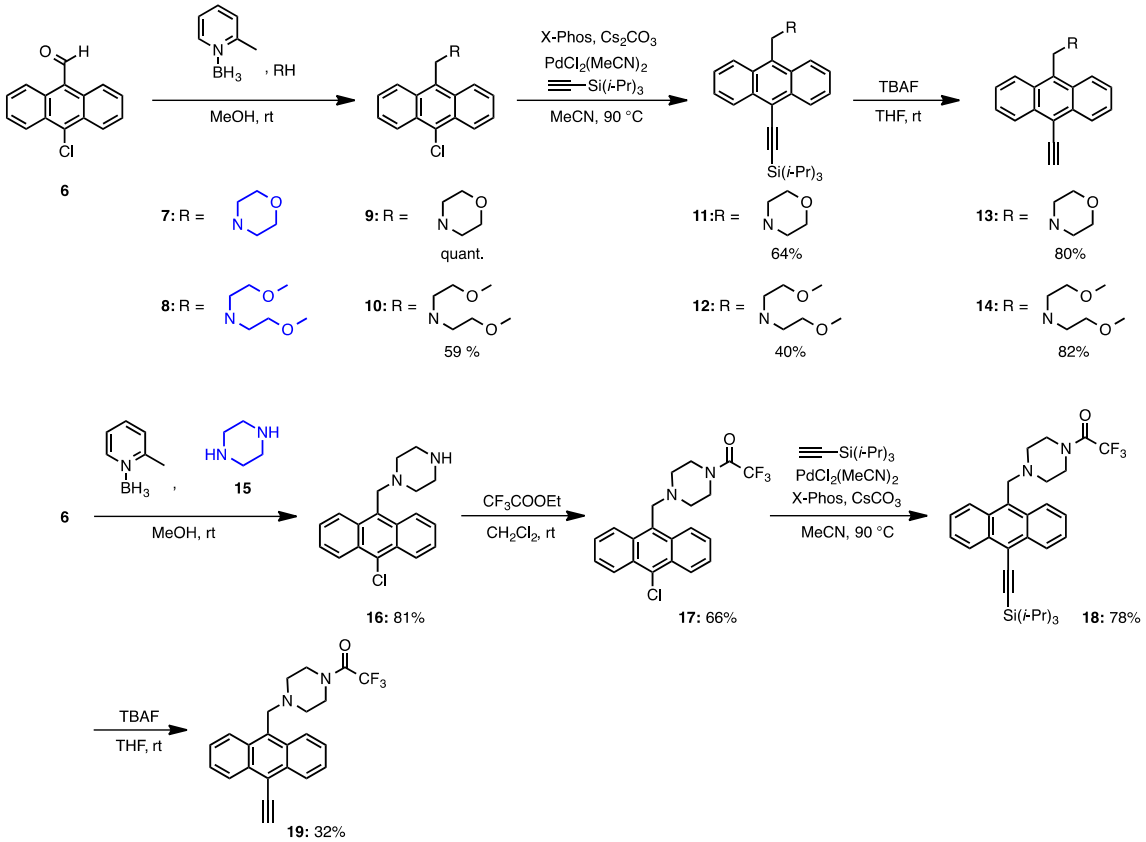


Fig. 2. Structures of the 6-modified 2′-deoxyadenosine derivatives.



Scheme 1.