Tetrahedron Letters 55 (2014) 94–97

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Design, synthesis and anti-influenza virus activities of terminal modified antisense oligonucleotides

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ARTICLE INFO

Article history:
Received 23 June 2013
Revised 21 October 2013
Accepted 27 October 2013
Available online 5 November 2013

Keywords:
Antisense oligonucleotides
Influenza virus
H1N1
Terminal modification

ABSTRACT

Four novel terminal modified antisense oligonucleotides (ODNs) were designed, synthesized and tested for their anti-influenza virus activity. Initial biological studies indicated that lipophilic and rimantadin emodificated Flutide exhibited more potent anti-H1N1 activity than Flutide. Among them, lipophilic modificated ODN (Flutide-**I**) showed the most antiviral activity. The EC_{50} value of Flutide-**I** for inhibiting H1N1 induced cytopathic effect (CPE) and H1N1 RNA were respectively (0.26 ± 0.16) μM and (0.11 ± 0.03) μM. The cytotoxicity of these compounds has also been assessed. No significant cytotoxicities were found for any of these compounds with the concentrations up to 20 μM.

Introduction

In 1978, Paul Zamecnik and Mary Stephenson reported the first experiments on antisense mechanisms of gene silencing, using short synthetic antisense oligonucleotides (ODNs) to inhibit replication of the Rous sarcoma virus by binding and blocking the action of 35s RNA, then increasing attention turned to the possible therapeutic applications of antisense technology.[1,2] In 1998, the first antisense drug, Vitravene (ISIS Pharmaceuticals Inc.), was approved for retinitis induced by cytomegalovirus.

In recent years, antisense ODNs have been applied to the treatment of a variety of diseases including viral infection,[3] tumor,[4] vessel restenosis,[5] fulminant septic shock,[6] asthma, and allergies.[7] In Jan 29th, 2013, the U.S. FDA approved the second antisense drug, Kynamro (mipomersen sodium, ISIS Pharmaceuticals Inc. and Genzyme Corp.) to treat inherited cholesterol disorder, which is an oligonucleotide inhibitor for homozygous familial hypercholesterolemia (HoFH).[8] Antisense ODN is designed to hybridize to a complementary target sequence of corresponding mRNA, which inhibits protein expression. Therefore antisense ODNs may display increase in affinity and selectivity for their nucleic acid targets compared with traditional drugs.[9,10]

Flutide (Scheme 1) is a 13-mer antisense phosphorothionate oligonucleotide (PS-ODNs), which is complementary to the 5′ terminal conserved regions of viral RNA found in almost all of the influenza A virus.[11] All of the nonbridging oxygen atoms in the phophodiester bond are replaced by sulfur in its structure. The introduction of phosphorothioate linkages into ODNs is primarily intended to enhance their nuclease resistance.[12] Previous studies have showed that Flutide exhibited the most potent antiviral activity in vitro and in vivo. It inhibited influenza virus A induced cytopathic effects in MDCK cells with the EC_{50} ranging from 2.2 to 4.4 μM. In the infected mouse model, prolonged mean survival days and declined virus titres in lung in the Flutide treatment groups compared with the infected control group, with a dose-dependent manner.[11]

Some reports have showed that terminal modification of ODNs facilitates the cellular import and increases the antiviral activity.[13,14] Therefore, as a continuous research program of our laboratory to improve the drugability of Flutide, we now report the design and synthesis of terminal modificated Flutide (Scheme 2) and their anti-influenza virus activity.

Results and discussion

Flutide and modified ODNs are solid phase synthesized with the phosphoramidite approach. The whole synthetic process has been completely automated with DNA synthesizer (ABI3900).

As shown in Scheme 3, 2-(4-aminobutyl)propane-1,3-diol **3** was prepared from 4-bromobutyronitrile **1** and diethyl malonate

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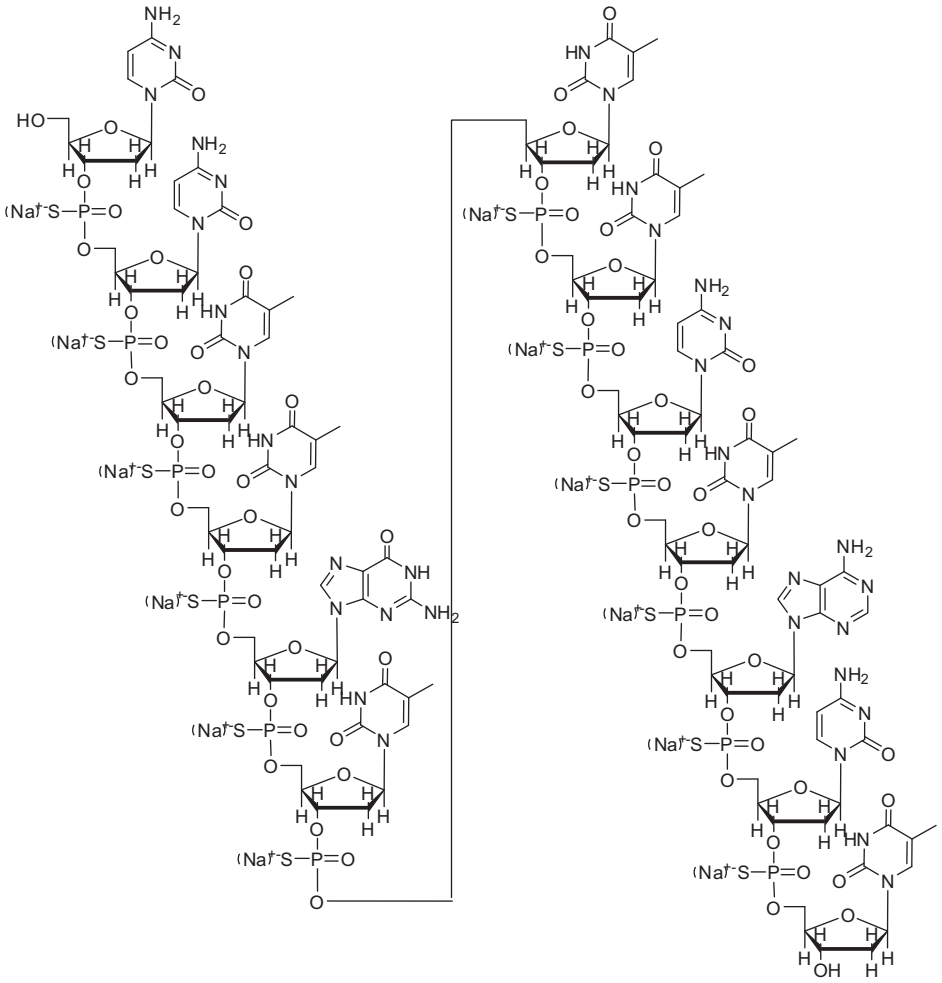
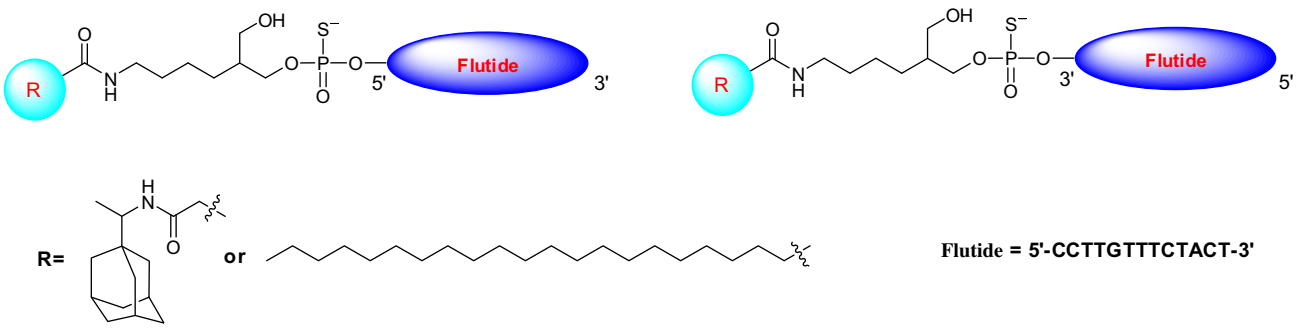
0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.10.129



Scheme 1. Structure of Flutide.



Scheme 2.

2 via condensation reaction and reduction reaction successively.[15] Treatment of behenic acid **4** and **3** with 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride in acetonitrile gave *N*-(6-hydroxy-5-(hydroxymethyl)hexyl)docosanamide **5** in 88% isolated yield. Subsequent selective protection of one primary hydroxy group of **5** with 4,4′-dimethoxytrityl group (DMT) provided **6** in 37% yield. Next, **6** was treated with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite ($((iPr_2N)_2POCH_2CH_2CN$) together with diisopropylammonium and tetrazolide in dry acetonitrile to afford 2-((bis(4-methoxyphenyl)(phenyl) methoxy)methyl)-6-docosanamidohexyl(2-cyanoethyl) diisopropylphos-phoramidite **7** in 78% isolated yield. Then, the terminal modified Flutide-**II** was synthesized on a 1.0 μmol scale by using **7**, as a kind of modified phosphoramidite, which coupled to the 5′ termini of Flutide following the standard PS-ODN synthesis process with the commercially DNA phosphoramidites (Sigma–Aldrich) and Unylinker™ 200 solid support (Nitto Denko Cor.). On the other hand, **6** was treated with butanedioic anhydride in tetrahydrofuran at ambient temperature for 12 h. Then NH_2 group of the Controlled Pore Glass (CPG) was added to the reaction system to afforded **8**, which was used as the solid support for the synthesis of Flutide-**I**. Rimantadine terminal modified