

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/lncn20>

A METHOD FOR SYNTHESIS OF AN ARTIFICIAL RIBONUCLEASE

Hans Åström^a & Roger Strömberg^b

^a Division of Organic and Bioorganic Chemistry, MBB, Scheele Laboratory, Karolinska Institutet, Stockholm, S-171 77, Sweden

^b Division of Organic and Bioorganic Chemistry, MBB, Scheele Laboratory, Karolinska Institutet, Stockholm, S-171 77, Sweden

Published online: 07 Feb 2007.

To cite this article: Hans Åström & Roger Strömberg (2001) A METHOD FOR SYNTHESIS OF AN ARTIFICIAL RIBONUCLEASE, Nucleosides, Nucleotides and Nucleic Acids, 20:4-7, 1385-1388

To link to this article: <http://dx.doi.org/10.1081/NCN-100002561>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4-7), 1385-1388 (2001)

A METHOD FOR SYNTHESIS OF AN ARTIFICIAL RIBONUCLEASE

Hans Åström and Roger Strömberg*

Division of Organic and Bioorganic Chemistry, MBB, Scheele Laboratory, Karolinska Institutet, S-171 77 Stockholm, Sweden

ABSTRACT

5-Amino-2,9-dimethyl-1,10-phenanthroline-oligonucleotide conjugates have been synthesized. A 2'-*O*-methyl octaribonucleotide carrying a 2'-aminoethoxymethyl linker in a central position was produced. Reaction of the amino-neocuproine phenyl carbamate with the fully deprotected oligonucleotide in aqueous solution gave virtually quantitative conversion into the conjugate. Preliminary cleavage studies in presence of zinc ions show nuclease activity towards RNA targets.

Oligonucleotide based artificial nucleases for hydrolytic cleavage of RNA have potential for use in the next generation of antisense reagents. Such artificial nucleases are generally designed to cleave target sequences using a tethered catalytic group (1). These compounds have the advantage that catalytic turnover can be achieved without the need of cellular enzymes, like RNase H. The catalytic group can be an acid-base catalyst or a metal chelate. In the present study conjugates of 5-amino-2,9-dimethyl-1,10-phenanthroline and a modified oligonucleotide have been synthesized and evaluated for use as artificial ribonucleases in the presence of Zn^{2+} . Use of the same phenanthroline derivative in a different oligonucleotide based artificial nuclease construct has recently been reported (2). The initial design is based on a 2'-*O*-methyl modified 8-mer (5'-GAGUACUC-3') oligonucleotide (**1**) carrying a 2'-aminoethoxymethyl linker in a central position (Fig. 1a). The amino linker is used to attach 5-amino-2,9-dimethyl-1,10-phenanthroline via a urea linkage (Fig. 1b).

*Corresponding author.

(a) Oligonucleotide Sequences

- (1) 5'-mGmAmGUxmAmCmCG-3'
 (2) 5'-CGG UAC UC-3'
 (3) 5'-CGG UAA CUC-3'

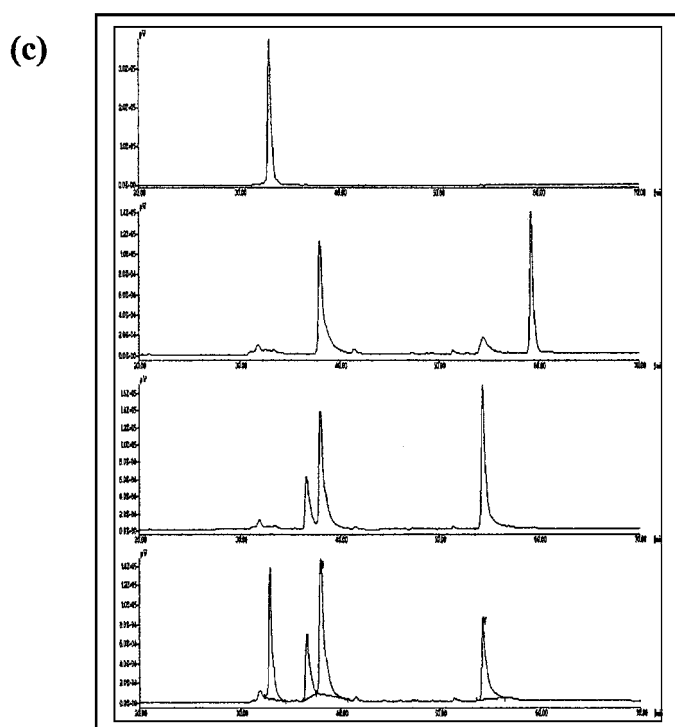
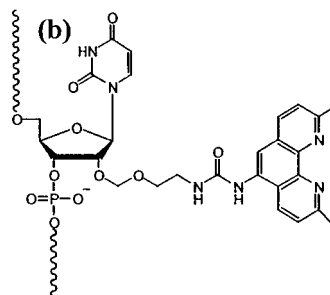
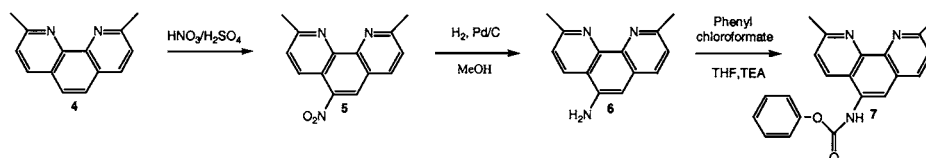


Figure 1. **b** shows a schematic presentation of the conjugate part of **1**. **a** shows the oligonucleotides used in this study, **2** and **3** are substrates for the nuclease. **c** shows HPLC analysis of the conjugation reaction: The top chromatogram is non conjugated. The second is reaction mixture without oligomer. The third spectra shows the crude reaction mixture after incubation for 2 hours. The bottom chromatogram is from mixing the above reaction mixture with non conjugated oligomer. The first peak in the third chromatogram was isolated and identified as the peak corresponding to **1**.

A synthetic route for the preparation of the nucleases has successfully been developed. The catalytic group could be conjugated in aqueous media to the already assembled and deprotected oligonucleotides in a virtually quantitative yield by reacting the free amino group of the linker with the phenyl carbamate of the amino phenanthroline derivative. Neocuproine (**4**) was first converted into the 5-NH₂-analogue (Scheme 1). 5-NO₂-2,9-dimethyl-1,10-phenanthroline (**5**) was



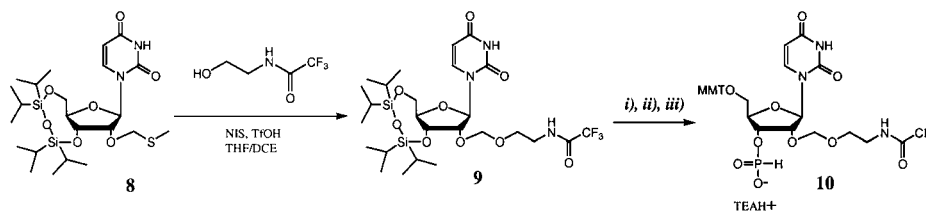


Scheme 1. Synthetic route to the neocuproine reagent.

synthesized from **4** using $\text{SO}_3/\text{H}_2\text{SO}_4$, HNO_3 , 168°C (**3**). The nitro derivative **5** was then reduced to 5-amino-2,9-dimethyl 1,10 phenanthroline (**6**) by catalytic hydrogenation on palladium/carbon. The amino derivative **6** was then subsequently converted into the phenyl carbamate **7** by reaction with phenyl chloroformate in THF in the presence of triethyl amine (Scheme 1).

The building block bearing the linker arm was synthesized from uridine (Scheme 2) via a previously developed route (Katcevic D., Rozners, E., Bizdena, E. and Strömberg, R., unpublished results). Uridine was first protected with the 1,1,3,3- tetraisopropylidisiloxyanylidene (TIPDS) group. Reaction of the 3',5'-TIPDS-uridine with DMSO/acetic anhydride/acetic acid (**4**) gave after silica gel chromatography and crystallization from hexane-ether the desired product **8**. Coupling of **8** with N-protected aminoethanol (made from reaction of aminoethanol with *S*-Ethyl trifluorothioacetate) using NIS/TfOH (**5**) activation gave the desired product **9**. The silyl protection was removed using fluoride ion and the deprotected compound was converted into building block **10** suited for use in the H-phosphonate method for oligonucleotide synthesis (**6**). This was achieved by standard monomethoxytritylation in pyridine followed by phosphorylation with the PCl_3 /imidazole reagent (**7**). The oligonucleotides were then made by machine assisted solid phase synthesis using a standard protocol.

The conjugation reaction was performed in sodium tetraborate buffer (pH 8.5) and seemed to be quantitative as judged from analysis by reversed phase HPLC (Fig. 1c). The oligonucleotide conjugate was purified by reversed phase HPLC giving homogeneous products and mass spectrometry analysis confirmed the identity of the conjugate.



i) $\text{Bu}_4\text{N}^+ \text{F}^-/\text{THF}$; ii) MMTCI/Pyridine; iii) (a) PCl_3 /imidazole/ Et_3N (b) water

Scheme 2. Coupling of protected aminoethanol to **5**.



The thermal stability of the 2'-*O*-Me modified oligonucleotide **1** was determined by means of thermal melting of complex with complementary sequence **2**. In order to determine the effect of the catalytic group on the duplex-stability, the melting temperature was checked both before and after conjugation with **7**. The melting point was determined to 44°C and 39°C respectively.

Kinetic cleavage studies have been initiated using the 5-aminoneocuproine-**1** nuclease to cleave complementary oligonucleotide substrate **2** and sequence **3** that contains an additional adenosine unit in the middle of the sequence. In both cases cleavage was detected in presence of 10-mM Zn²⁺ ions at pH 7. In the case of sequence **3** the cleavage appears to be more sequence selective. In the case of 5-aminoneocuproine-**1** nuclease cleaving substrate **2** the estimated first order rate constant is $2 \times 10^{-4} \text{ min}^{-1}$.

EXPERIMENTAL

Synthesis of the 5-aminoneocuproine-1 conjugate. Carbamate **7** (250 µg, 0.73 µmol) was dissolved in 14 µl dry DMSO. To this solution was added: 7 µl H₂O, 75 µl Sodium tetraborate buffer (0.1-M, pH 8.5) and finally a 4 µl (37 nmol) solution of oligonucleotide **1**. The vial containing the reaction mixture was agitated, 2 µl aliquotes were withdrawn from the reaction mixture, filtrated, diluted to 100 µl water and analysed with reversed phase (RP) HPLC. The reaction was incubated overnight although it appeared complete in 2 h. The reaction mixture was then filtered and purified on RP HPLC. 18.3 nmol product corresponding to a yield of 50% was isolated. Both analytical and preparative HPLC was performed on a Hypersil ODS column (5 µm, 4.6 × 25 mm) using 5 min isocratic elution with 0.1-M triethylammonium acetate (*aq.*), then a linear gradient to the same buffer containing 20% MeCN in 35 min and finally from 20 to 50% MeCN in 20 min. Flow rate was 1 ml/min and temperature 50°C.

REFERENCES

1. Trawick, B.N.; Daniher, A.T.; Bashkin, J.K. *Chem. Rev.* **1998**, 98, 939–960.
2. Putnam, W.C.; Bashkin, J.K. *Chem. Comm.* **2000**, 767–768.
3. Smith, G.F.; Cagle, F.W. JR. *J. Org. Chem.* **1947**, 781–784.
4. Pojer, P.M.; Angyal, S.J. *Tetrahedron Lett.* **1976**, 3067–3068.
5. Veeneman, G.H.; Van der Marel, G.A.; Van der Elst, H.; Van Boom, J.H. *Tetrahedron* **1991**, 47, 1547–1562.
6. (a) Garegg, P.J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, 27, 4051–4054. (b) Garegg, P.J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, 27, 4055–4058. (c) Froehler, B.C.; Matteucci, M.D. *Tetrahedron Lett.* **1986**, 27, 469–472.
7. (a) Garegg, P.J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chemica. Scripta* **1986**, 26, 59–62. (b) Stawinski, J.; Strömberg, R.; Thelin, M.; Westman, E. *Nucleic Acids Res.* **1988**, 16, 9285–9298.



Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

[Order now!](#)

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081NCN100002561>