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Efficient site-specific cleavage of RNA using a terpyridine–copper(II) complex joined to a 2′-*O*-methyloligonucleotide by a non-flexible linker

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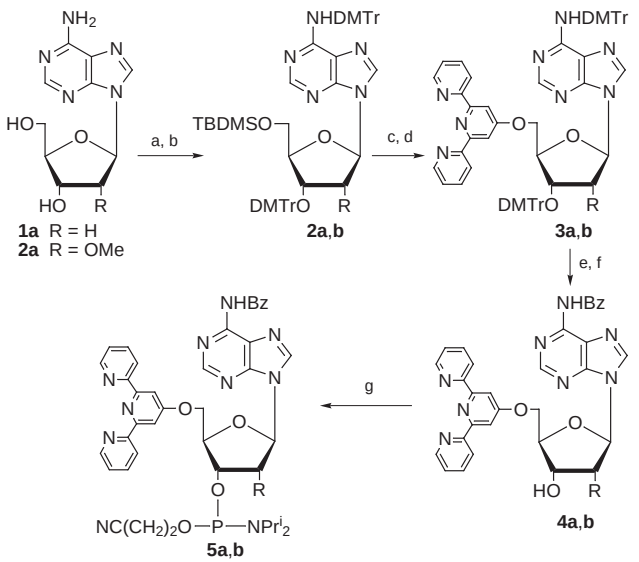
A terpyridine–Cu(II) complex conjugated to an antisense 2′-*O*-methyloligonucleotide at the 5′-end cleaved RNA predominantly at the site opposite the 5′-end in moderate yield, but the cleavage yield increased by more than two-fold when a 2′-*O*-methyloligonucleotide was allowed to bind to the single-stranded region of the RNA-antisense complex.

Recently, chemical agents that cleave RNA site-specifically without enzymatic assistance have been developed.[1] This is potentially important in antisense chemotherapy because cleavage of a specific mRNA will lead to loss of its function.[2] These agents can also be useful for studies on structure–function relationships of native RNAs.[3] One relatively active class of RNA cleavers is metal complexes covalently linked to oligonucleotides; the cleavage mechanism may involve transphosphorylation promoted by the metal. Complexes, such as terpyridine–Cu(II),[4,5] macrocyclic terpyridine derivative–Ln(III),[6,7] texaphyrin–Ln(III),[8,9] and iminodiacetate–Ln(III),[10] have been attached by a flexible linker to DNA oligomers for sequence-specific recognition. Here, we describe a new RNA cleaver with an alternative arrangement, consisting of a 2′-*O*-methyl RNA oligomer, as an efficient RNA hybridization probe,[3,11] and a terpyridine–Cu(II) complex directly attached to the 5′-end of the 2′-*O*-methyloligomer. We also demonstrate RNA cleavage by the cleaver in the presence of a cleavage facilitator.

For oligonucleotide synthesis, 5′-*O*-terpyridyl 3′-phosphoramidite derivatives of 2′-deoxyadenosine **1a** and 2′-*O*-methyladenosine **1b** were prepared, using standard methods for all steps (Scheme 1). The synthetic route involving *O*-terpyridylation[12] is easy and applicable to the other nucleosides. The two building units **5a**,**b** were used for the preparation of 5′-end-modified 2′-*O*-methyloligonucleotides.



Scheme 1 *Reagents and conditions*: for **5b** (all reactions were carried out at room temperature). a, TBDMSCl (1.2 equiv.), imidazole (2.4 equiv.), DMF, 1.5 h, 77%; b, DMTrCl (2.6 equiv.), pyridine, 23 h, 77%; c, Bu_4NF (1.2 equiv.), THF, 30 min, 99%; d, 4′-chloro-2,2′ : 6′,2″-terpyridine (1.2 equiv.), KOH, DMSO, 44 h, 90%; e, 80% $MeCO_2H$ aq, 4 h, 100%; f, successive steps: i, TMSCl (3 equiv.), pyridine, 30 min; ii, BzCl (5 equiv.), 3.5 h; iii, H_2O, 5 min; iv, 28% NH_3 aq, 30 min, 64% (overall); g, 2-cyanoethyl diisopropylchlorophosphoramidite (1.2 equiv.), diisopropylethylamine (3 equiv.), CH_2Cl_2, 50 min, 50%. DMTr = 4,4′-dimethoxytrityl.

Cleavage reactions of RNA oligomers with the terpyridine–Cu(II)-oligomer conjugates were carried out with the 5′[^{32}P]-end-labeled RNA target in a buffer at 45 °C for 20 h. Representative examples of an analysis of the cleavage products and all results are indicated in Figs. 1 and 2, respectively. Cleavage of the RNA 24-mer (R24) with the 5′-*O*-Cu(II)–terpyridyl-2′-deoxyadenosine-linked 12-mer (dA*12) occurred predominantly between U12–U13, in which U12 is positioned at the end of the 12-mer recognition region [Figs. 1 and 2(a)].† The extent of specific cleavage was 18% after 20 h [Fig. 2(c)]. Minor cleavage occurred at U13–U14 in *ca.* 1% yield. For the corresponding 5′-*O*-Cu(II)–terpyridyl-2′-*O*-methyladenosine-conjugate (Am*12), similar cleavage results were obtained. An RNA 21-mer with no sequence similarity to R24 was also specifically cleaved by a dA*-13-mer at a predetermined site, 5′U–A3′ (26% yield, data not shown).

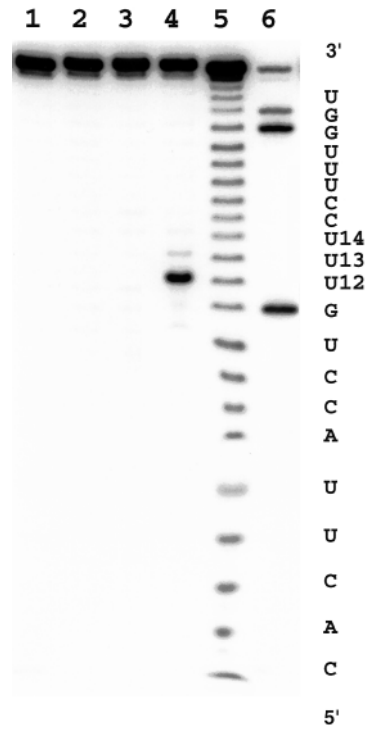
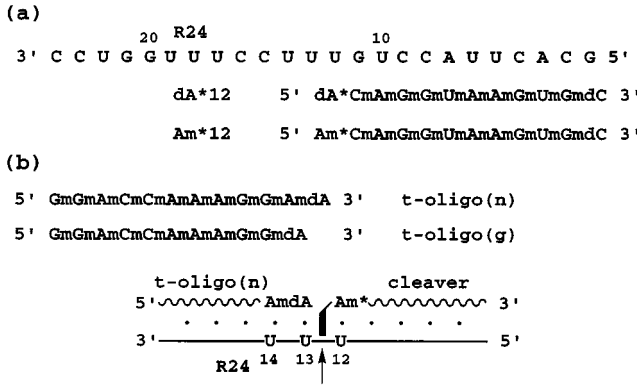


Fig. 1 Autoradiogram of products from the reaction of the labeled target R24 with the agent dA*12 after 20% denaturing polyacrylamide gel electrophoresis. The reaction was carried out for 20 h at 45 °C in a total volume of 10 μl containing: 0.1 M $NaClO_4$, 20 mM HEPES (pH 7.5), 0.1 μM RNA, 1 μM agent, and 1 μM $CuCl_2$. Lane 1: R24; lane 2: R24 + free terpyridine–Cu(II) complex + control 12-mer without terpyridine moiety; lane 3: R24 + dA*12 + EDTA (10 mM); lane 4: R24 + dA*12; lanes 5 and 6: bicarbonate and ribonuclease T1 sequencing reactions, respectively.



reactions	yields of cleavage between U12-U13	
	dA*12	Am*12
without t-oligo	18%	21%
with t-oligo(n)	27%	48%
with t-oligo(g)	17%	24%
(minor cleavage[a]	3%	6%)

Fig. 2 (a) Sequences of the RNA substrate and terpyridine–Cu(II)-linked oligomer agents. Nm refers to a 2′-*O*-methylnucleoside residue, and the asterisk indicates the site of the linked terpyridine moiety. (b) Sequences of the tandem 2′-*O*-methyloligomers, and schematic representation of RNA cleavage by the use of Am*12 and the t-oligo(n) (the arrow shows the cleavage site). The terpyridine–Cu(II) moiety is indicated as a black rectangle. All 2′-*O*-methyloligomers used in this study had a 2′-deoxynucleoside residue at the 3′-end. (c) Yields of site-specific cleavage of R24 by agents with or without tandem oligonucleotides.
[a] The minor cleavage site is U13–U14 site, and other reactions also gave this cleavage, but in yields of less than 1%.

For induction of RNA cleavage, the terpyridine–Cu(II) complex attached to the non-flexible linker should be close to the RNA strand. To explore the spatial orientation of the terpyridine moiety in the oligonucleotide hybrid, we prepared a terpyridine-linked duplex with a self-complementary sequence (dA*CmAmGmCmUmGmUm) and examined its melting temperature [conditions: 0.1 M $NaClO_4$, 10 mM Na phosphate (pH 7.5), 10 μM oligonucleotides, 12 μM $CuCl_2$]. It was found that the T_m value (72 °C) was very high, as compared with that (52 °C) for the control duplex without terpyridine moieties in the presence of 12 μM $CuCl_2$. These results may mean that the terpyridine moiety interacts with the end of the hybrid in an end-capping manner [Fig. 2(b)].[13]

To explore efficient RNA cleavage, which may be affected by the environment around the terpyridine–Cu(II) moiety in the RNA–agent hybrid, we carried out the RNA cleavage reaction in the presence of a 2′-*O*-methyl RNA complementary to the RNA single strand region adjacent to the terpyridyl conjugated 12-mer. The adjacent oligonucleotide was positioned in two ways: first, to provide a nick, and second a gap at the terpyridine site. In each reaction, with or without the tandem oligonucleotide [t-oligo(n) or t-oligo(g), Fig. 2(b)], Am*12 was more effective than dA*12 [Fig. 2(c)]. The cleavage efficiency for the reaction with the gapped tandem sequences was similar to that for the reaction without the additional oligonucleotide, but the cleavage site-specificity was lower. On the other hand, the reactions using the nicked tandem sequences increased the cleavage efficiency. Moreover, when Am*12 instead of dA*12 was used, the cleavage yield was 48%.‡ The spatial orientation of the terpyridine moiety probably became more suitable for RNA cleavage by the presence of the nick-forming tandem sequences and the 2-*O*-methylribose linker.

The present RNA cleaver has the metal complex at the end of the 2′-*O*-methyloligonucleotide. On the other hand, RNA cleaving metal complexes that were attached within DNA oligomers have been reported.[4,5,7,9] Some of the agents were designed to remain in a local region (such as a bulge) containing the cleavage site loose[14] while hybridized with the target RNA. This kind of agent is expected to be developed into an artificial ribonuclease that acts with catalytic turnover. Our findings, including the results on the nick-containing RNA hybrid, provide valuable information for the design of catalysts and the creation of more efficient cleavers.

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Notes and references

† For the reaction, the agent and $CuCl_2$ were directly added to the RNA solution. The addition after premixing both compounds gave similar results for the cleavage. We have used 10 molar equivalents of the terpyridine–Cu(II) agent and found that 2 molar equivalents were sufficient for the reaction. The use of such small amounts of cleavage agents has not been reported.

‡ Recently Daniher and Bashkin reported RNA cleavage using a terpyridine–Cu(II) complex attached to a flexible linker on an abasic site analog within DNA oligomers.[5] Our cleavage yield using the 2′-*O*-methyl facilitator and our data for the site-specificity of the RNA cleavage seem to be comparable with the reported data.

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