

AT-CuAAC Synthesis of Mechanically Interlocked Oligonucleotides

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Cite This: *J. Am. Chem. Soc.* 2020, 142, 5985–5990



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ABSTRACT: We present a simple strategy for the synthesis of main chain oligonucleotide rotaxanes with precise control over the position of the macrocycle. The novel DNA-based rotaxanes were analyzed to assess the effect of the mechanical bond on their properties.

Mechanically interlocked molecules (MIMs)¹ based on oligonucleotides² predate even the early synthetic work of Wasserman, Harrison, and Schill;³ catenated DNA, which arises during DNA replication and is managed by topoisomerase enzymes,⁴ was observed as early as 1967 by Vinograd and co-workers,⁵ and threaded structures play an important role in the operation of some DNA polymerase enzymes.^{6,7} To date, artificial oligonucleotide-based MIMs have been produced using DNA self-assembly approaches,⁸ including origami methods.⁹ Although this approach allows the production of complex architectures and stimuli responsive systems, the threaded structures produced are relatively large (ring sizes are typically >100 base pairs), and the sequences assembled are not of direct biological relevance. DNA-based MIMs containing non-nucleotide macrocycles have not been reported, presumably as the majority of the methods developed for the synthesis of rotaxanes are not well-suited to the production of functional interlocked DNA; passive template methods¹ would require significant modification of the sugar-phosphate backbone, whereas hydrophobic threading-based approaches are unsuitable due to the hydrophilicity of oligonucleotides.

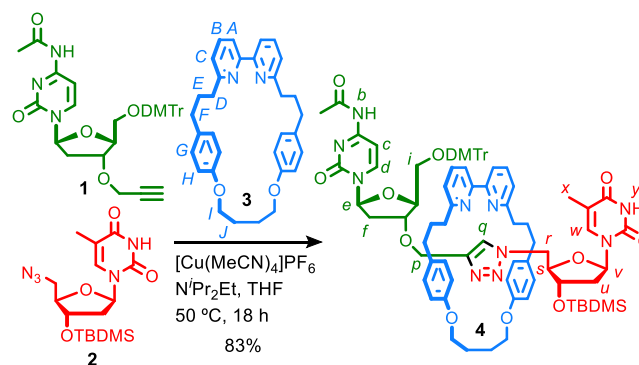
Tavassoli, Brown and co-workers have developed biocompatible triazole linkages to replace a native phosphodiester bond in an oligonucleotide strand, and have shown this non-natural modification to be fully biocompatible in bacterial and human cells.¹⁰ This “click-DNA ligation” approach overcomes the size limitations of automated DNA synthesis by allowing longer oligonucleotides to be synthesized by CuAAC ligation of ~100 base fragments functionalized with alkyne or azide handles, for example, in the one-pot synthesis of epigenetically decorated, biocompatible, triazole-linked genes.¹¹ Click-DNA ligation also presents an additional opportunity; Leigh’s active template¹² Cu-mediated alkyne/azide cycloaddition (AT-CuAAC)¹³ reaction permits the simultaneous installation of a triazole moiety and the formation of a mechanical bond.¹⁴

Here we report the synthesis of biologically relevant DNA rotaxanes by combining Goldup’s small macrocycle¹⁵ modification of the AT-CuAAC reaction with Tavassoli and Brown’s click-DNA approach. The mechanical bond significantly alters the supramolecular and biological properties of

the oligonucleotide. Our results suggest that the mechanical bond can be used to tailor the behavior of biocompatible DNA.

As the AT-CuAAC reaction had not previously been applied to substituted nucleotides, we began our study by demonstrating the formation of a [2]rotaxane by reaction of propargyl cytosine **1** and azido thymine **2**, models of the chain termini in the click-DNA ligation process, in the presence of macrocycle **3**¹⁶ (Scheme 1). Pleasingly, under our standard AT-CuAAC

Scheme 1. Synthesis of Rotaxane 4 from Cytosine-Derived Alkyne 1 and Thymine-Derived Azide 2^a



^aDMTr = C(Ph)(4-(OMe)-C₆H₄)₂, TBDMS = ^tBuMe₂Si.

conditions,¹⁵ rotaxane **4** was produced in excellent isolated yield (83%). Mass spectrometry confirmed the expected protonated molecular ion of **4** ($m/z = 1470$), and the ¹H NMR spectrum of the purified product displayed the expected features for such interlocked species; triazole proton H_g appears 1.5 ppm higher in rotaxane **4** than the noninterlocked axle, consistent with the expected C–H···N hydrogen bond to

Received: February 11, 2020

Published: March 10, 2020

the bipyridine unit (Figure 1).¹⁵ In addition, many macrocycle resonances, including H_A, H_B, H_C, H_F, and H_G, which appear as

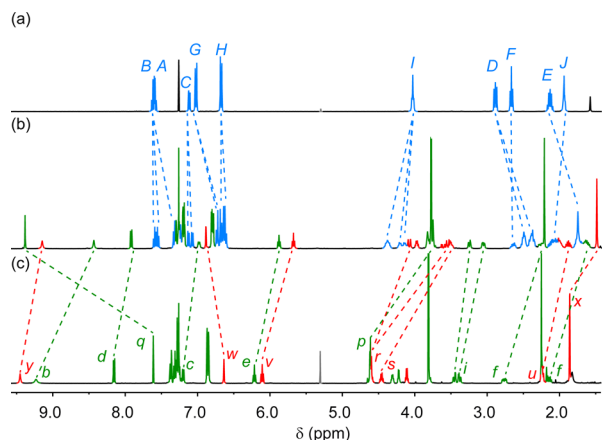
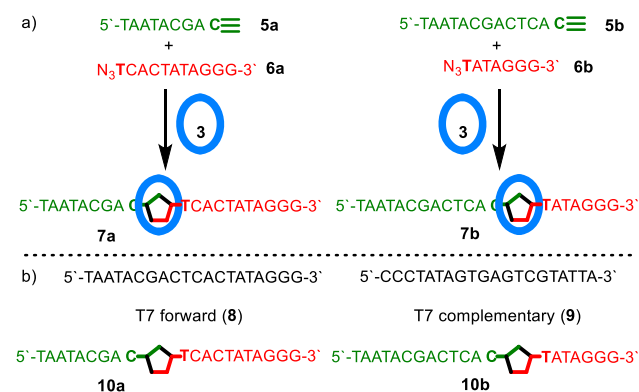


Figure 1. ¹H NMR (400 MHz, CDCl₃, 298 K) with selected signals assigned (see Scheme 1 for atom labels) of (a) macrocycle 3; (b) rotaxane 4; (c) the corresponding noninterlocked axle.

single signals in noninterlocked 3, are split into two signals as the bilateral symmetry of the ring is lifted in the chiral interlocked product.^{15a,17}

Having demonstrated the synthesis of simple triazole-linked dinucleotide [2]rotaxane 4 we turned our attention to the challenge of synthesizing a longer interlocked oligonucleotide using the AT-CuAAC approach. For our proof of concept study, we selected the 20 base T7 promoter sequence, widely used in a variety of biological applications.¹⁸ Alkyne 5a and azide 6a were synthesized using standard solid phase techniques, and their AT-CuAAC coupling was optimized by systematic modification of the conditions reported for click-DNA ligation (see Supporting Information). Ultimately, reaction of 5a and 6a in the presence of macrocycle 3 using THF–H₂O (1:1) as the solvent, CuSO₄/Na ascorbate as the source of Cu^I, and NⁱPr₂Et as base to accelerate the reaction gave T7 rotaxane 7a as the sole product (no noninterlocked axle 10a was detected by LC-MS analysis) in 83% isolated yield after HPLC purification (Scheme 2). T7-based rotaxane

Scheme 2. (a) AT-CuAAC Synthesis of T7-Rotaxanes 7a and 7b;^a (b) Control Compounds T7 Forward, T7 Complementary, and Axles 10a and 10b



^aReagents and conditions: (i) macrocycle 3 (21 equiv), CuSO₄ (10 equiv), Na ascorbate (50 equiv), ⁱPr₂EtN (10 equiv), THF–H₂O (1:1), rt, 16 h.

7b, which differs in the position of the mechanical bond along the DNA backbone, was produced under the same conditions from alkyne 5b and azide 6b in 90% isolated yield. LC-MS analysis confirmed the purity and identity of both interlocked products. Native oligonucleotides T7 forward (8) and noninterlocked triazole axes 10a and 10b were synthesized separately as control compounds. Rotaxanes 7 and axes 10 display significantly different HPLC retention times (~8.5 vs ~7.5 min, respectively).

To evaluate the effect of the mechanical bond on duplex formation, rotaxanes 7 were annealed with the T7 complementary (9) oligonucleotide and the resulting mixture was analyzed by circular dichroism (CD) spectroscopy (Scheme 3). Whereas the native T7 forward (8) and noninterlocked

Scheme 3. Annealing of Rotaxanes 7, T7 Forward (8), and Axles 10 with T7 Complementary (9) and Their Melting Temperature (T_m) Determined by CD Spectroscopy^a

Entry	Forward strand	T_m / °C
1	8	47.0
2	7a	no duplex
3	7b	no duplex
4	10a	38.0
5	10b	39.5

^aAnnealing conditions: 10 μM, buffer–H₂O (8:1), 95–15 °C over 40 min.

triazole-containing oligonucleotides 10 displayed the expected CD signals at rt between 180 and 200 nm associated with expression of helicity in a DNA duplex,^{19,20} rotaxanes 7 display weak CD signals between 180 and 200 nm (Figure 2). Furthermore, raising the temperature slowly to “melt” the duplex led to the expected sharp decrease in the CD signal associated with duplex formation in the case of samples derived from 8 and 10, whereas no sharp transition associated with

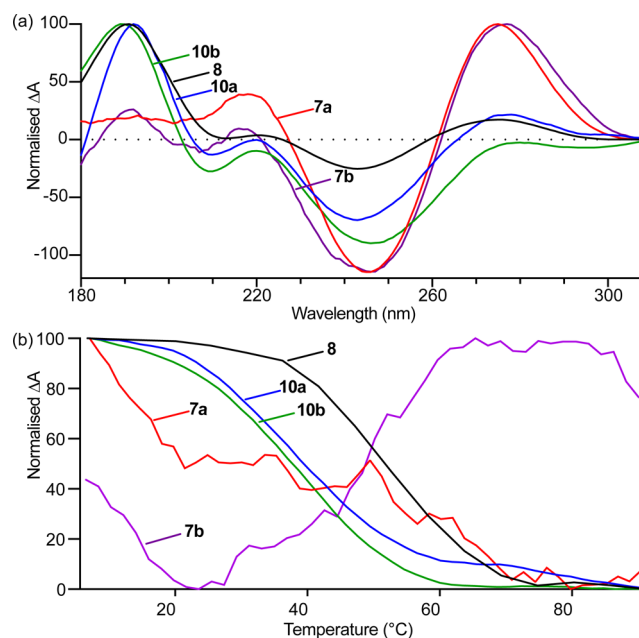


Figure 2. (a) Normalized CD spectra (25 °C) of rotaxanes 7, T7 forward (8), and axles 10 annealed with T7 complementary (9). (b) Normalized CD melting curves (190 nm) of rotaxanes 7, T7 forward (8), and axles 10 annealed with T7 complementary (9).

duplex disassembly was observed for rotaxanes 7.¹⁹ Taken together, these results imply that rotaxanes 7 do not hybridize to form a DNA duplex with their complementary strand, and thus DNA hybridization is completely suppressed by the mechanical bond.

The failure of rotaxanes 7 to form a duplex with their complementary strand suggests that the mechanical bond acts to “cage” the oligonucleotide. Caged DNA and RNA based on covalent modification of the native base pairs have been developed to allow selective control of biological function.²¹ Typically, however, multiple points of modification are required for efficient suppression of hybridization,²² whereas in the case of rotaxanes 7 it appears that a single modification is sufficient to achieve complete suppression of duplex formation.

To demonstrate a potential biological consequence of this result, we examined the behavior of rotaxanes 7a and 7b when used as primers for PCR. The native T7 forward primers and noninterlocked triazole-containing oligonucleotides 10a and 10b were used as positive controls. Both of these control primers successfully amplified a 1000 bp fragment from a template plasmid at various annealing temperatures (55 °C, 41 °C, and 32 °C) to give a single band of the expected molecular weight (Figure 3). However, in line with the lack of duplex

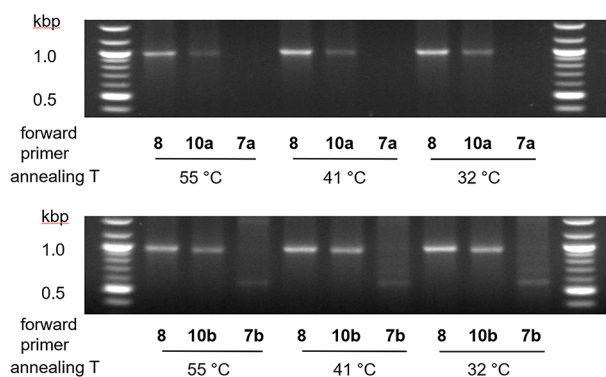


Figure 3. (a) Gel analysis of the PCR amplification products of T7 forward (8), rotaxanes 7, and axles 10 at annealing temperatures 55 °C, 41 °C, and 32 °C.

formation suggested by the melting experiments, when oligonucleotide rotaxanes 7a and 7b were used as the forward primer for PCR amplification, no amplification was observed even at the relatively low annealing temperature of 32 °C (Figure 3). Based on these results, the mechanical bond in rotaxanes 7a and 7b effectively suppresses the ability of the interlocked oligonucleotide to function as a primer for T7 polymerase.

In conclusion, we have demonstrated that the CuAAC approach used in click-DNA ligation can be readily extended to the active template manifold in order to generate rotaxanes based on biocompatible triazole-linked oligonucleotides. Furthermore, whereas the noninterlocked axles are able to form a duplex with their complementary strand and also function as primer sequences for the amplification of an oligonucleotide, the interlocked products are not; duplex formation and PCR amplification are completely suppressed by a single macrocycle encircling the axle, demonstrating that the mechanical bond is an efficient modification for the “caging” of oligonucleotides. Although interlocked molecules are well-known as components of artificial molecular machines,²³

interest in their biological applications has grown in recent years,²⁴ including as pro-drugs,²⁵ sensors,²⁶ and delivery agents for biologically active molecules.²⁷ Based on our preliminary results, mechanical bonding has a key role to play in the development of artificial stimuli responsive DNA for real time chemical biology investigation of gene expression and protein function.²¹ Future work will focus on the development of cleavable macrocycles that can be removed in response to external or biological stimuli to reactivate oligonucleotide bioactivity and extending our approach to longer oligonucleotides and plasmids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.0c01670>.

Full experimental details and characterization data for all novel compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of Southampton, EPSRC (EP/L016621/1), BBSRC (BB/J001694/2), and ERC (724987) for financial support. M.G. thanks the EPSRC for a Doctoral Prize. A.A.J. is a Marie-Curie Fellow (798304). S.M.G. is a Royal Society Wolfson Research Fellow.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published ASAP on March 20, 2020, with uncorrected versions of Scheme 1 and Figures 1 and 2. The corrected version of the paper was reposted on March 23, 2020.