AT-CuAAC Synthesis of Mechanically Interlocked Oligonucleotides

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Supporting Information placeholder

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, overcome 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.9 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 (Scheme 1). Pleasingly, under our standard AT-CuAAC 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 Hq appears 1.5 ppm higher in rotaxane 4 than the non-interlocked axle, consistent with the expected C-H···N hydrogen bond to the bipyridine unit.15 In addition, many macrocycle resonances, including HA, HB, HC, HD, and Hc, which appear as single signals in non-interlocked 3, are split into two signals as the bilateral symmetry of the ring is lifted in the chiral interlocked product.15a,17

Scheme 1. Synthesis of rotaxane 4 from cytosine-derived alkyne 1 and thymine-derived azide 2.

Having demonstrated the synthesis of simple triazole-linked di-nucleotide [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 optimized by systematic modification of the conditions reported for click-DNA ligation (see ESI). 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 Pr2EtN as base to accelerate the reaction, gave T7 rotaxane 7a as the sole product (no non-interlocked axle 10a was detected by LC-MS analysis) in 83% isolated yield after HPLC purification. T7-based rotaxane 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 non-interlocked triazole axles 10a and 10b were synthesized separately as control compounds. Strikingly, rotaxanes 7a and 7b display significantly different HPLC retention times (~8.5 vs ~7.5 min respectively), which is surprising given that they differ only by inclusion of macrocycle 3, a relatively small change compared with the size of a 20-base oligonucleotide.

To evaluate the effect of the mechanical bond on duplex formation, rotaxanes 7 were annealed with the T7 complementary (g) oligonucleotide and the resulting mixture was analyzed by circular dichroism (CD) spectroscopy. Whereas the native T7 forward (8) and non-interlocked 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,18,19 rotaxanes 7 display weak CD signals between 180 and 200 nm. 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 duplex disassembly was observed for rotaxanes 7.21 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.

Scheme 3. Annealing of rotaxanes 7, T7 forward (8) and axles 10 with T7 complementary (g) and their melting temperature (Tm) determined by CD spectroscopy.

4Annealing conditions: 10 μM, buffer-H2O (8:3), 95 – 15 °C over 40 min.

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 non-interlocked axle.

Scheme 2. a) AT-CuAAC synthesis of T7-rotaxanes 7a and 7b. b) Control compounds T7 forward, T7 complementary and axles 9a and 9b.

Reagents and conditions: (i) macrocycle 3 (21 equiv.), CuSO4 (10 equiv.), Na ascorbate (50 equiv.), Pr2EtN (10 equiv.), THF-H2O (1:1), rt, 16 h.
quired for efficient suppression of hybridization, whereas in the case of rotaxanes 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 and when used as a primer for PCR. The native T4 forward primers and non-interlocked triazole-containing oligonucleotides and 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 formation suggested by the melting experiments, when oligonucleotide rotaxanes and were used as a 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 and effectively suppresses the ability of the interlocked oligonucleotide to function as a primer for T7 polymerase.

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

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 non-interlocked 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, interest in their biological applications has grown in recent years, including as pro-drugs, sensors, and delivery agents for biologically active molecules. 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. Future work will focus on the development of cleavable macrocycles that can be removed in response to external or biological stimuli to reactivated oligonucleotide bioactivity and extending our approach to longer oligonucleotides and plasmids.

ASSOCIATED CONTENT

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bio-compatible click-DNA

active template
"click"-DNA ligation

CuI

selective
high yield

mechanically silenced click-DNA

no duplex formation
PCR suppressed

anneal

anneal