Biocompatible artificial DNA linker that is read through by DNA polymerases and is functional in *Escherichia coli*

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A triazole mimic of a DNA phosphodiester linkage has been produced by templated chemical ligation of oligonucleotides functionalized with 5′-azide and 3′-alkyne. The individual azide and alkyne oligonucleotides were synthesized by standard phosphoramidite methods and assembled using a straightforward ligation procedure. This highly efficient chemical equivalent of enzymatic DNA ligation has been used to assemble a 300-mer from three 100-mer oligonucleotides, demonstrating the total chemical synthesis of very long oligonucleotides. The base sequences of the DNA strands containing this artificial linkage were copied during PCR with high fidelity and a gene containing the triazole linker was functional in *Escherichia coli*.

replicated in bacteria | triazole DNA backbone | click chemistry | CuAAC reaction

Solid-phase DNA synthesis (1, 2) is an advanced technology that has led to pioneering discoveries in biology and nanotechnology (3–8). Although automated solid-phase phosphoramidite synthesis is highly efficient, the accumulation of modifications (mutations) and failure sequences caused by side-reactions and imperfect coupling imposes a practical limit of around 150 bases on the length of oligonucleotides that can be made. Consequently very long synthetic oligonucleotides are not suitable for use in biological applications that require sequence fidelity, so combinations of shorter sequences are normally used in PCR-mediated gene assembly (9, 10). This enzymatic method of DNA synthesis has the intrinsic limitation that site-specific chemical modifications can only be introduced in the primer regions of the resulting constructs. Certain unnatural analogues can be inserted throughout the PCR amplicon via modified dNTPs, but this process is essentially uncontrolled and does not allow combinations of different modifications to be incorporated at specific loci. Therefore, for biological studies, important epigenetic and mutagenic bases such as 5-methyl dC, 5-hydroxymethyl dC and 8-oxo dG are normally put into short oligonucleotides and subsequently inserted into larger DNA strands by enzymatic ligation. Templated enzymatic ligation of oligonucleotides can be used to produce large DNA fragments, but this is best carried out on a small scale. In addition, some modified bases are not tolerated by ligase enzymes. Enzymatic methods of gene synthesis are extremely important in biology, but a purely chemical method for the assembly of large DNA molecules would be an interesting and valuable addition to current tools, with the advantages of scalability, flexibility, and orthogonality.

It has proved challenging to achieve clean and efficient chemical ligation of canonical DNA, although significant progress has been made using cyanogen bromide as a coupling agent (11, 12). An interesting alternative approach is to design a chemical linkage that mimics the natural phosphodiester bond, and that can be formed in high yield in aqueous media from functional groups that are orthogonal to those naturally present in DNA. This goal has been partly achieved by the reaction between oligonucleotides with 3′-phosphorothioate and 5′-tosylate or iodide (13, 14). Three key requirements of our strategy are however, not fully satisfied by the above; the use of functional groups that are highly stable in aqueous media, the ability to selectively initiate the ligation reaction only when participating oligonucleotides have been hybridized to complementary splints (to arrange the DNA strands in the desired order by templated preassembly), and the creation of a very stable backbone linkage. We recently described a high-yielding DNA ligation method (click ligation) based on the CuAAC reaction (15, 16). Click chemistry has been used extensively in the nucleic acids field (17–21) and it fulfils all the above criteria (22–24). We demonstrated that although the DNA triazole linkage is read through by PCR (Fig. 1*B*) (25), amplification of the resulting modified DNA template caused the loss of one nucleotide at the site of click ligation. The consistently observed deletion mutation in the resulting PCR products indicated that this artificial DNA linkage is not an adequate mimic of a phosphodiester group, suggesting that it is unlikely to behave like its natural counterpart in vivo. This triazole DNA linker has another limitation: synthesis of the required azide precursor oligonucleotides relies on a combination of reverse phosphoramidite and phosphotriester chemistry. These procedures impose limitations on the length and purity of oligonucleotides that can be conveniently prepared, making adoption of the methodology in commercial DNA synthesis laboratories unlikely. In addition, the chemistry is not readily adaptable to the synthesis of oligonucleotides containing both azide and alkyne functionalities (one at each terminus), essential building blocks for gene assembly by multiple chemical ligation reactions. Despite these constraints, the previous study indicates that there is scope to produce a truly biocompatible DNA linkage, particularly considering the flexibility of the CuAAC reaction and the wide variety of alkynes and azides that may be utilized (21). We now describe the synthesis and properties of a newly designed triazole phosphodiester surrogate whose precursor oligonucleotides can be readily prepared by standard phosphoramidite methods, and that is functional both in vitro and in *Escherichia coli*. It is a unique example of a biocompatible artificial DNA linkage that can be formed efficiently by chemical ligation.

Results and Discussion

Synthesis and Assembly of Azide/Alkyne Oligonucleotides. When incorporated into a PCR template the T-triazole-T of our previous

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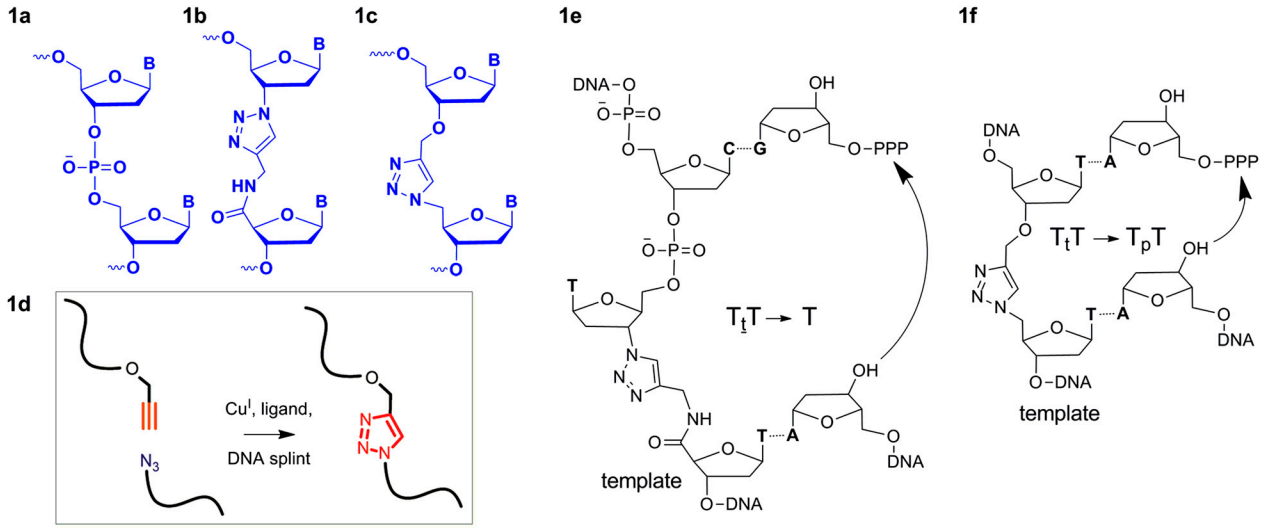

Fig. 1. DNA linkage structures. In 1a, canonical DNA; 1b, previous triazole DNA analogue (25); 1c, biocompatible triazole analogue; 1d, click ligation to produce triazole DNA mimic 1c; and 1e polymerases read through 1b using only one of the two thymines as a template base; i.e., $T_tT \rightarrow T$ (t = triazole). In 1f, PCR copies the base sequence around the unnatural linkage 1c correctly.

artificial DNA linkage (Fig. 1*B*) was copied by thermostable polymerases as a single T (25). A possible explanation for this is the presence of the rigid amide bond, which may cause the triazole T in the template to turn away from the growing DNA strand during replication. The lack of a 3′-oxygen atom and a 5′-methylene group as recognition sites might also make this linkage a poor polymerase substrate. With these considerations in mind an improved triazole linkage was designed without an amide bond and with 5′-methylene group and 3′-oxygen atom to better resemble a natural phosphodiester (Fig. 1*C*). This unique linkage also has the considerable advantage of being constructed from oligonucleotides made entirely by the phosphoramidite method, one bearing a 5′-azide functional group and the other a 3′-alkyne. The functionalized resin required for the solid-phase synthesis of oligonucleotides terminating with 3′- propargyl ^{Me}dC (cytosine equivalent, Fig. 2) was prepared from thymidine as previously described (26). A polystyrene support was used in this case, to achieve high coupling yields and produce 100-mer oligonucleotides of the purity required for efficient click ligation. DNA

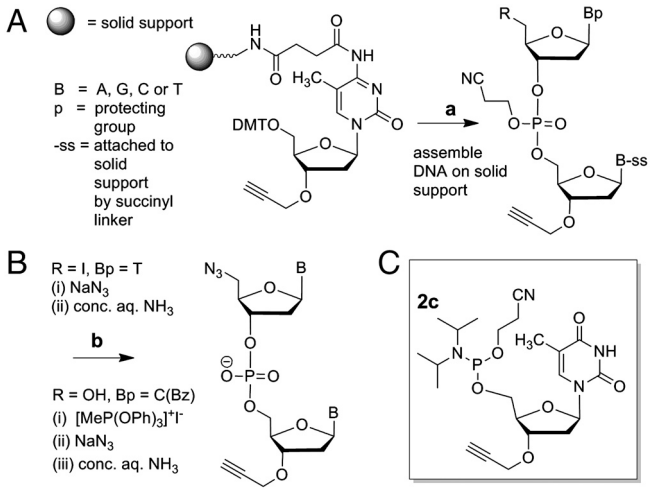

Fig. 2. Synthesis of alkyne/azide oligonucleotides for use in click ligation and cyclization. (*A*) Assembly of 3′-alkyne oligonucleotide. (*B*) Conversion to 5′-azide. Oligonucleotides can be made with 5′-azide, 3′-alkyne or both. A dinucleotide is shown for clarity but the reactions have been carried out on oligonucleotides up to 100-mer in length. (*C*) The 3′-Propargyl dT introduced as final addition in reverse phosphoramidite assembly of DNA.

strands containing 3′-propargyl dT were made from reverse phosphoramidites that required the synthesis of monomer 2c. The 5′-azide group was introduced in a 2-stage process (Fig. 2); the 5′-OH group of a normal support-bound oligonucleotide was first converted to 5′-iodo by reaction with methyltriphenoxyphosphonium iodide (27) (for oligonucleotides with 5′-dT this was simplified by direct incorporation of 5′-iodo thymidine phosphoramidite), then the resultant 5′-iodo oligonucleotides were reacted with sodium azide to complete the transformation (28). Oligonucleotides functionalized with both 3′-alkyne and 5′-azide were made by performing oligonucleotide synthesis on 3′-propargyl ^{Me}dC resin then converting the 5′-terminus to azide as described above. In this study the bases on either side of the triazole linkage are thymine and cytosine (or 5-methylcytosine). This is an adequate combination for the synthesis of any large DNA strand by click ligation, but in future it should be possible to use the same methodology for other combinations of nucleosides.

Amplification of Click DNA by Thermostable Polymerases. To investigate the compatibility of linkage 1c with thermostable polymerases, three 81-mer DNA templates were synthesized, each containing a single triazole linkage. We found that PCR of these constructs produced amplicons that were faithful copies of the original sequence (Figs. S1 and S2), with the T_tT, T_tC, $^{Me}C_tT$, and $^{Me}C_tC$ linkages being read through accurately (t = triazole 1c). It is possible however, that PCR amplification of the chemically modified DNA might appear to be efficient even if readthrough of the artificial linkage is a rare event. The ability of DNA polymerases to replicate through the triazole linkage was therefore evaluated more rigorously by linear copying of an 81-mer (ODN-8; Table S1) using Large Klenow fragment. The reaction was efficient and the full length product was obtained in less than 5 min (Fig. S3).

Application of click DNA ligation to the synthesis of large linear DNA constructs requires oligonucleotides that are functionalized at both termini. We therefore evaluated the simultaneous ligation of three 11-mer oligonucleotides in the presence of a complementary 41-mer splint. The click ligation reaction was clean (Fig. S4) and the product was characterized by electrospray (ES) mass spectrometry (calc. 10,064, found 10,064; Table S2). The integrity of the terminal alkyne and azide is essential for efficient click ligation, so it was important to show that the large numbers of repeated steps employed in the synthesis of long oligonucleotides do not destroy these functional groups. This was

confirmed by successfully cyclising a 100-mer with 5′-azide and 3′-alkyne functionalities. The reaction proceeded smoothly in the absence of a complementary template oligonucleotide and the product was characterized by gel-electrophoresis and mass spectrometry (Fig. 3 *A* and *B* and Fig. S5). Under similar conditions enzymatic cyclization failed, whereas in templated mode both the chemical and enzymatic cyclization reactions were successful (Fig. S6).

To demonstrate the utility of click ligation for the assembly of large DNA molecules, a 210-mer PCR template was assembled from three 70-mers (Fig. S7), as well as a 300-mer from three 100-mer oligonucleotides. The oligonucleotides were designed to have an even distribution of A, G, C, and T bases and to be devoid of secondary structure. The ligation products were purified by gel-electrophoresis and used as templates in PCR, after which the amplified regions were cloned, sequenced, and found to be correct (Fig. 4). Thermostable polymerases with or without proofreading activity (*Pfu* and GoTaq respectively) read through the sequence around the click linkers to give the expected amplicons (Fig. S2). In this study a total of four different base stacking steps (Y_tY, all possible combinations of pyrimidines) on either side of the triazole were examined in several different tetramer sequences (Table S1), and in all cases (134 clones) the bases encompassing the triazole linkages were replicated correctly. In addition to PCR, we were able to carry out rolling circle amplification (RCA) (29, 30) of a cyclic 100-mer containing a triazole linkage, using the highly processive phi29 polymerase. The cyclic template was produced in an intramolecular click ligation reaction of a 5′-azide-3′-alkyne oligonucleotide (ODN-30; Table S1). An essentially identical profile of phi29 RCA products was obtained from both normal and triazole cyclic templates (Fig. 3*D*), and we were even able to obtain long RCA products using GoTaq polymerase under standard PCR cycling conditions, by repeated read-through of the triazole linkage in a short timescale (Fig. S8). The amplified RCA product was probed with a fluorescent HyBeacon (31) to confirm that it was a true copy of the original template rather than a nonspecific amplification product (Fig. S9).

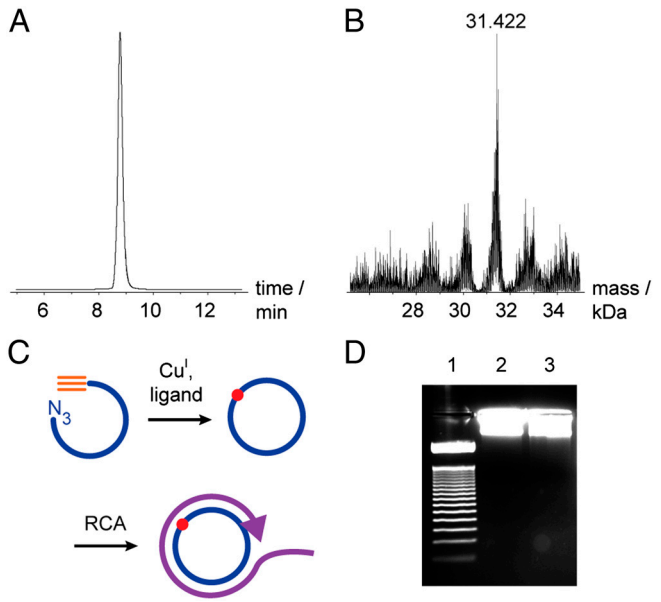

Fig. 3. Cyclization and RCA of 5′-azide-3′-alkyne 100-mer. (*A*) Reversed-phase HPLC (UV abs at 260 nm) and (*B*) mass spectrum (ES$^-$) of cyclic 100-mer ODN-31, required; 31.423 kDa, found 31.422 kDa. (*C*) Schematic of RCA reaction. (*D*) RCA product from cyclic 100-mers using phi29 DNA polymerase. Lane 1; 50 bp DNA ladder, lanes 2 and 3; RCA of cyclic triazole ODN-31 and cyclic normal ODN-49 respectively.

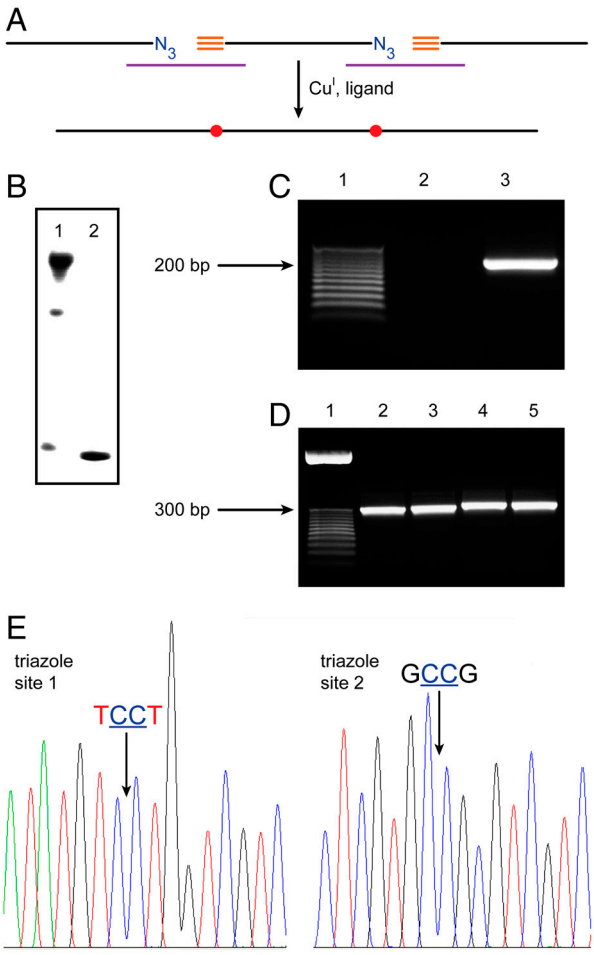

Fig. 4. PCR amplification of 210- and 300-mer click-ligated triazole DNA templates. (*A*) Schematic representation of click ligation of three oligonucleotides. (*B*) Click ligation reaction: Lane 1; crude reaction mixture to synthesize 210-mer template from three 70-mers, lane 2; starting oligonucleotide ODN-16 (8% polyacrylamide gel). (*C*) PCR using 210-mer triazole template. Lane 1; 25 bp DNA ladder, lane 2; control PCR without click-ligated template, lane 3; PCR using 210-mer triazole template. (*D*) PCR using 300-mer triazole template. Lane 1; 25 bp DNA ladder, lanes 2, 3; PCR using short primers ODN-26 and ODN-27, Lanes 4, 5; PCR using long primers ODN-28 and ODN-29 (2% agarose gels with ethidium staining). (*E*) Sequencing data from 300-mer triazole amplicon showing that the base sequence of the template was replicated faithfully at the two ligation sites.

Biocompatibility of the Click Linker in *E. coli*. Following the successful in vitro experiments we next investigated the biocompatibility of the modified DNA within the cellular machinery of *E. coli* (Fig. 5) by constructing a plasmid containing a triazole linkage in each strand of its antibiotic marker gene. The triazole linkages were introduced via modified PCR primers that amplify a portion of the TEM-1 ß-lactamase (BLA) gene between the ScaI and PvuI restriction sites (Fig. S10). PCR with these primers yielded a product matching the middle section of BLA, containing $^{Me}C_tC$ near the 3′ terminus of each strand. Electrophoresis of the amplicon showed it to be of the expected size and identical in length to that from the control PCR carried out with unmodified primers (Fig. S11). The products of both PCR reactions (using unmodified and modified primers) as well as a plasmid containing the BLA gene (T7-Luciferase control, Promega Inc.) were digested with ScaI and PvuI restriction endonucleases. The digested plasmid (now lacking the region between ScaI and PvuI in its BLA gene) was gel-purified to remove the insert and undigested/singly digested plasmid, and treated with shrimp alkaline phosphatase to remove the phosphate monoesters from the 5′-termini to

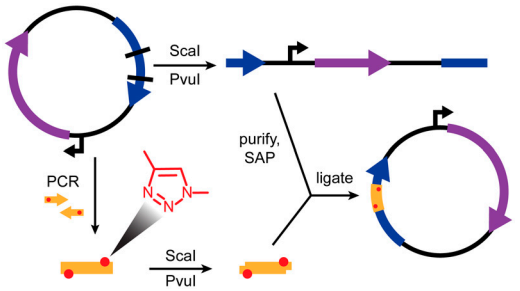

Fig. 5. Assembly of a T7-Luciferase control plasmid containing click-DNA within its BLA gene. A region corresponding to the central part of BLA was PCR-amplified using oligonucleotide primers (ODN-39, ODN-41) containing triazole linkage 1c. The PCR product was ligated into the digested plasmid to give an intact construct containing triazole linkages on each strand of its BLA gene.

prevent self-ligation. The digested PCR products were then ligated into the linearized plasmid backbone via the matching ScaI and PvuI sites using T4 DNA ligase. A control ligation reaction was also set up containing water in place of the insert to measure the level of ampicillin resistance arising from the presence of partially digested or undigested backbone. The resulting ligation mixtures were transformed into *E. coli* (NEB 5α) and grown on LB-agar plates containing 100 μg/mL of ampicillin (21 plates of each type). After overnight incubation at 37 °C the number of colonies from the triazole plasmids was 96.5% of the native, whereas the negative control was only 1.6% (Fig. 6). We isolated plasmid and sequenced the BLA gene from 50 of the surviving colonies on both the positive control and the triazole DNA plates. In all cases the base sequence at the $^{Me}C_tC$ linkage was copied correctly (Fig. S12).

The survival and growth of colonies containing a triazole-modified antibiotic marker gene suggests that the sequence around the triazole linkage is amplified correctly by the *E. coli* polymerases. However, viability might also be maintained if the

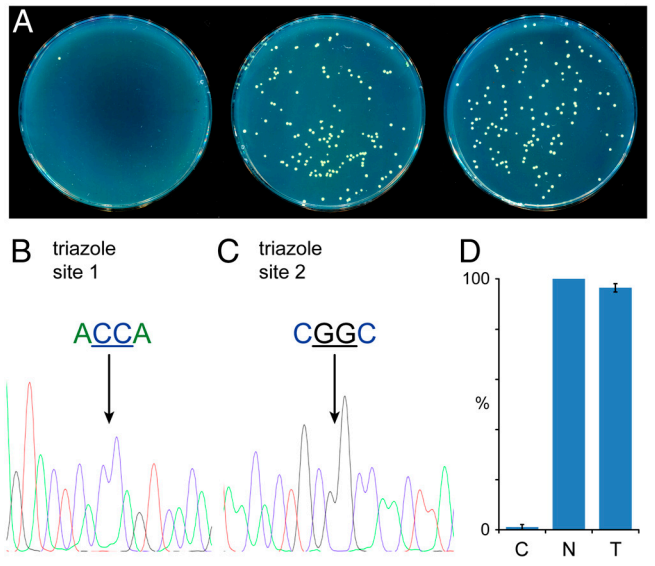

Fig. 6. Biocompatability of click DNA in *E. coli*. (*A*) The plate on the left is the negative control (no insert), the middle plate contains transformants of plasmids with the triazole DNA insert in its BLA gene (127 colonies), and the plate on the right is the native plasmid (129 colonies). Twenty-one replicates of each plate were performed. (*B*, *C*) Sequencing of the BLA gene from colonies in the triazole DNA plates. In *C*, the $^{Me}C_tC$ is contained on the complementary strand, therefore appearing as GG. (*D*) Comparison of colony growth in the control (C), native (N), and triazole (T) plates. Triazole plates contained 96.5% of the colonies in the native plates (S.D. = 1.6%) whereas the negative control was 1.1% (S.D. = 1.0%).

region surrounding the triazole modification was excised by the cellular DNA repair machinery via nucleotide excision repair (NER) and replaced by a phosphodiester linkage. This possibility was investigated using a UvrB-deficient strain of *E. coli* (JW0762-2) (32). UvrB is a central component of NER, interacting with UvrA, UvrC, UvrD, DNA polymerase I, and DNA (33) during excision-repair (34, 35). If the biocompatibility of the click-DNA linker was a consequence of NER, repair-deficient colonies would not survive on selective media when transformed with the triazole plasmid. Transformation of the repair-deficient strain of *E. coli* with the triazole plasmid gave 93% of the number transformed with the native plasmid (Fig. S13), and sequencing the BLA gene from 21 of the colonies revealed that the region around the triazole linkage was copied correctly in all cases (Fig. S14). This strongly supports the hypothesis that NER does not make a significant contribution to the biocompatibility of the triazole linkages.

Rationale for Biocompatibility of Triazole Linkage in DNA. The ability of DNA polymerases to accurately synthesize a complementary copy of an artificial DNA linkage that bears limited structural resemblance to a natural phosphodiester may seem surprising. However, the X-ray structure of the Klenow fragment of Taq polymerase (Klentaq-1) with double-stranded DNA at its active site (36) provides some insight into the underlying mechanism of this phenomenon. In this structure there are several polar interactions between the enzyme and the phosphodiester groups of the DNA template strand that are consistent with hydrogen bonding (Fig. 7*A*). As the polymerase passes through the chemically modified template-primer complex, only one of the ten template nucleotides bound to the enzyme at any given time can encompass a triazole. Hence a maximum of only two interactions can be disrupted by the modification. In addition, some enzyme binding at the triazole site could still occur, as the triazole moiety has a large dipole moment and well-characterized hydrogen bond acceptor capacity (37, 38). The requirement for dynamic and nonspecific binding between DNA and the enzyme might also explain why the presence of triazole linkage 1c does not compromise fidelity during PCR amplification. A similar picture of enzyme template binding emerges from the structure of DNA bound to Taq polymerase (39), a version of the enzyme that has 3′-exonuclease activity.

We postulate that linkage 1c with its 3′-oxygen, 5′-methylene and greater conformational flexibility is a closer analogue of a natural phosphodiester than 1b. In contrast to 1c, it is apparent that triazole 1b alters the characteristics of the DNA sufficiently to prevent faithful replication. The thymine base on the 5′-side of the triazole may not be presented at the polymerase domain in a suitable orientation to base pair with the incoming dATP, so the only option is for replication to continue from the next available template base (Fig. 1*E*). In addition, linkage 1b is by no means an obvious phosphodiester surrogate in terms of H-bonding acceptor capacity, so its binding to the polymerase may be compromised. The normally favored *trans*-configuration at the amide bond, and the extended rigidity of this linkage, may not allow the N2 and N3 atoms of the triazole to substitute for phosphodiester oxygen atoms (Fig. 7*D*). Regardless of the detailed mechanisms, our results indicate that the artificial DNA linker is remarkably biocompatible, and investigations are underway to solve the high-resolution structure of a DNA duplex containing this triazole linkage and determine its effects on DNA conformation and dynamics. Other DNA backbone mimics have been made (40, 41), but in these constructs the entire DNA strand was modified, precluding their use in PCR or any biological application requiring enzymatic processing. Nevertheless, these studies point to other interesting triazole analogues that could in principle be incorporated into DNA via chemical ligation as discrete units and investigated as potential biocompatible linkages.

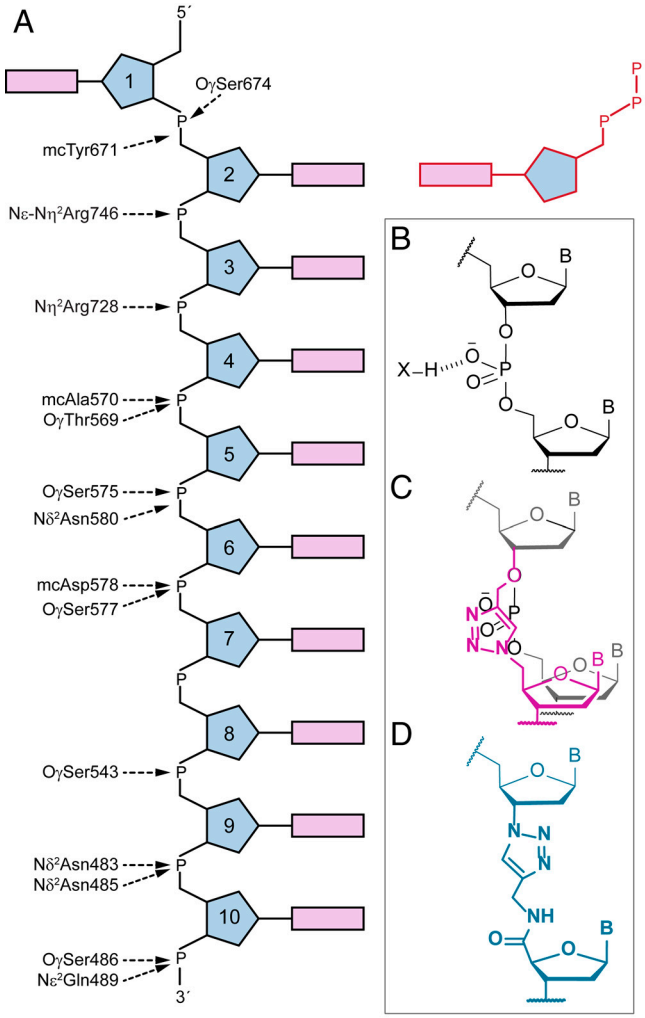

Fig. 7. Taq polymerase primer-template dNTP closed complex (36). (*A*) Schematic of interactions between the phosphodiester linkages of the DNA template and amino acids of the enzyme. Only template strand is shown. mc = main chain. (*B*) Canonical DNA; the majority of the interactions with the polymerase involve the branched phosphate oxygen atoms, few if any involve bridging oxygen atoms. (*C*) Overlay of canonical DNA and triazole linkage 1c. The N2 and N3 atoms of triazoles are good hydrogen bond acceptors (37, 38) and in principle they could substitute for the phosphate oxygen atoms. (*D*) Triazole linkage 1b showing the *trans*-configuration of the amide, with N2 and N3 of the triazole facing into the helix. Linkage 1b is significantly more rigid than 1a and 1c.

Conclusion

Clean and efficient methods of linking DNA strands are of great value in biology and nanotechnology. Chemical ligation is particularly interesting as it can be carried out on a large scale under a variety of conditions, as it does not depend on the use of enzymes. We have developed a triazole DNA linkage that can be created with high efficiency using the CuAAC reaction. The chemistry to synthesize the individual alkyne and azide containing DNA fragments is straightforward and is compatible with standard automated phosphoramidite oligonucleotide synthesis, and the entire DNA assembly procedure is simple and can be carried out routinely in service laboratories. The methodology has been used to synthesize oligonucleotides up to 300 bases in length, and when such constructs are used as PCR templates the entire sequence including the bases on either side of the triazole linkage is copied correctly. Hence, one could contemplate using click-DNA ligation for the assembly of synthetic genes on a large scale. The observation that a gene containing the triazole linkage is functional in *E. coli* is significant and could enable a number of applications. For example, incorporation of site-specific base analogues could facilitate the study of DNA replication, repair or epigenetic gene regulation, and chemical modifications such as fluorescent dyes could be included for in vivo imaging.

Materials and Methods

All oligonucleotide sequences are given in Table S1.

PCR and Sequencing of Triazole DNA Templates. ***PCR using GoTaq DNA polymerase.*** PCR products from 81-mer, 210-mer and 300-mer templates were generated using GoTaq DNA polymerase with 4 μL of 5× buffer (green buffer) in a total reaction volume of 20 μL with 5 ng of the DNA template, 0.5 μM of each primer, 0.2 mM dNTP and 0.5 unit of GoTaq. The reaction mixture was loaded onto a 2% agarose gel in 1 X Tris/Borate/EDTA buffer (TBE). PCR cycling conditions: 95 °C (initial denaturation) for 2 min then 25 cycles of 95 °C (denaturation) for 15 s, 54 °C (annealing) for 20 s and 72 °C (extension) for 30 s. 5 X Promega green PCR buffer was provided with the enzyme (containing Tris.HCl, KCl, 7.5 mM $MgCl_2$, pH 8.5) to give a final Mg^{2+} concentration of 1.5 mM.

PCR using Pfu DNA polymerase. PCR product from ODN-08 (81-mer C-triazole-C template) was generated using 2 μL of 10× buffer in a total reaction volume of 20 μL with 5 ng of the DNA template, 0.5 μM of each primer, 0.2 mM dNTP and 1.0 unit of *Pfu* DNA polymerase. (10× reaction buffer = 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1.0% Triton® X-100 and 1 mg/mL nuclease-free BSA.) PCR cycling conditions: 95 °C (initial denaturation) for 2 min then 25 cycles of 95 °C for 15 s, 54 °C for 20 s, and 72 °C for 30 s. This was followed by one cycle of 72 °C for 2 min.

Sequencing of clones from the PCR product of 81-mer, 210-mer, and 300-mer triazole templates. The PCR products were prepared as above using GoTaq or *Pfu* DNA polymerase and purified on a 2% agarose gel followed by extraction using QIAquick Gel Extraction kit Cat. No. 28704. The purified PCR products were then cloned and sequenced by the automated fluorescent Sanger method: 10 clones for ODN-25 (300-mer with two $^{Me}C_tC$ linkages), 50 clones for ODN-19 (210-mer with two $^{Me}C_tT$ linkages), 40 clones for ODN-08 (81-mer with $^{Me}C_tC$ linkage), 17 clones for ODN-07 (81-mer with T_tC linkage), and 17 clones for ODN-06 (81-mer with T_tT linkage). ODN-08 (81-mer with $^{Me}C_tC$) was amplified using both GoTaq and *Pfu* DNA polymerases, and 20 clones of each were sequenced. The polymerases read the sequence around the triazole linkages correctly for all 134 sequences.

Assessing the Biocompatibility of the Triazole DNA Linkage in *E. coli*. *PCR of BLA fragment with click primers.* The region between the ScaI and PvuI sites of BLA was amplified by PCR with GoTaq DNA polymerase using the click-linked oligonucleotides ODN-39 and ODN-41, 10 μL of 5× buffer in a total reaction volume of 50 μL with 1 ng of the DNA template, 1 μM of each primer, 0.2 mM dNTP and 1 unit of GoTaq. The reaction was repeated with normal oligonucleotides (no triazole linker). The reaction mixtures were loaded onto a 2% agarose gel in 1× Tris/acetate/EDTA buffer (TAE); both reactions gave products of identical size (Fig. S11). PCR cycling conditions were: 94 °C (initial denaturation) for 1.5 min then 35 cycles of 94 °C (denaturation) for 30 s, 46.5 °C (annealing) for 30 s and 72 °C (extension) for 30 s. The reaction was held at 72 °C for 5 min after the 35 cycles.

Restriction digestion of PCR product and vector. The PCR products were digested with ScaI HF and PvuI restriction endonucleases (NEB, Cat. No. R3122 and R0150) according to the manufacturer's protocol and was purified using QIAquick PCR purification kit (QIAGEN, Cat. No. 28106). The Luciferase T7 control plasmid (Promega, Cat. No. L4821) was also digested with ScaI HF and PvuI, and treated with thermosensitive alkaline phosphatase (Promega, Cat. No. M9910) to remove the 5′-phosphate groups from the linearized plasmid DNA, thus preventing recircularization during ligation. The linear plasmid was gel-purified using QIAquick gel extraction kit (QIAGEN Cat. No. 28706) to remove the undigested plasmid and the excised fragment.

Ligation reactions and transformation into E. coli. The digested PCR products (triazole and normal) and linearized plasmid were ligated for 16 h at 15 °C (total volume 10 μL, 1:3 vector:insert ratio) using T4 DNA ligase (Promega, Cat. No. M1801). Negative control ligations were set up as above, using water instead of insert. Five microliters of each ligation mixture was transformed into chemically competent *E. coli* (NEB 5α, NEB, Cat. No. C2992H) using the standard protocol. Transformants were recovered in 895 μL of SOC at 37 °C

with shaking for 1 h. One hundered microliters of each recovery solution was spread onto LB agar plates and incubated at 37 °C overnight. Colonies were counted using a Gel Doc XR+ system and Quantity One Software (both from BioRad Laboratories). The above procedure was repeated for the UvrB-deficient *E. coli* strain [JW0762-2, Coli Genetic Stock Center (CGSC), Cat. No. 8819] that was supplied by the CGSC at Yale University.

Sequencing of the BLA gene. Fifty colonies were picked from plates containing the plasmids with the triazole DNA insert in its BLA gene and 50 were picked from the positive control plates (normal BLA gene). The colonies were grown overnight in LB and the plasmids from each culture were isolated using QIAprep Spin miniprep kit (QIAGEN, Cat. No. 27106). They were then sequenced by the automated fluorescent Sanger method. We did not observe mutations in the region between the ScaI and PvuI sites in any of the plasmids. For the experiment on DNA repair, 21 colonies were sequenced from repair-deficient *E. coli* strain JW0762-2 and all sequences were found to be correct (Fig. S14).

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