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COMMUNICATION

Solid phase strain promoted "click" modification of DNA *via* [3 + 2]-nitrile oxide–cyclooctyne cycloadditions†

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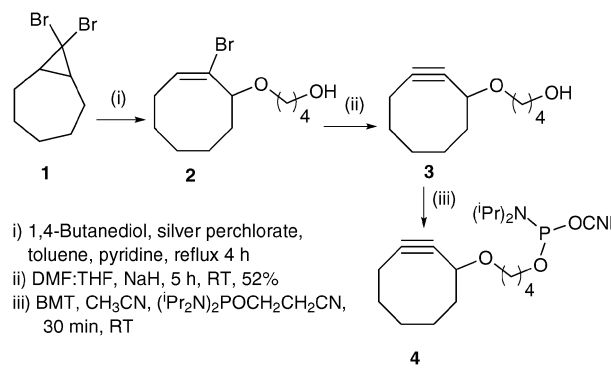
Rapid, catalyst free, solid phase modification of DNA by strain promoted cyclooctyne–nitrile oxide click chemistry is reported; the reaction is characterised by mild conditions, occurring in an aqueous environment under atmospheric conditions at room temperature and is complete in 10 minutes.

Chemically modified oligonucleotide-based drugs are promising therapeutic candidates,[1] however clinical applications are limited by poor cellular uptake.[2] The conjugation of oligonucleotides to molecules expected to facilitate their internalization, *e.g.* cell penetrating peptides or lipids, offers an attractive way to combat these shortcomings.[3] The Cu(I) catalysed azide and alkyne cycloaddition (CuAAC) reported by Meldal *et al.*[4] and Sharpless *et al.*[5] has been extensively used in synthetic chemistry and in chemical biology.[6,7] It is a high yielding and efficient reaction, however, for certain applications the requirement for the catalyst can be limiting.[6] The added metal can be cytotoxic[8] and can upset the metabolic balance of the systems under study, thus, a need exists to expand the field of metal free biocompatible chemistry. Approaches to-date include the photoinduceable cycloadditions of tetrazines[9b] or nitrile imines to alkenes,[9a] and the [3 + 2]-azide–cyclooctyne cycloaddition developed by Bertozzi *et al.*[10,11] and Boons *et al.*[12] The latter, exploiting the intrinsic ring strain of cyclooctynes[13] is a powerful reaction, yet it can require several hours to reach completion at room temperature. In one elegant example Pezacki *et al.* circumvents this problem by employing nitrones as more reactive dipole partners.[14] We and others have recently discovered nitrile oxide click conjugation to alkenes[15,16] and to terminal alkynes[17–19] as a highly effective approach to chemical modification of oligonucleotides. Nitrile oxides are reactive 1,3-dipoles, and in the absence of an effective dipolarophile side reactions, including dimerisation leading furoxans, 1,2,4-oxadiazole-4-oxides or 1,4,2,5-dioxadiazines could be problematic.[20] However, we anticipated high chemoselectivity for the desired cycloaddition with a very reactive strained cycloalkyne partner. Whilst there are numerous applications of cyclooctynes in the context of biological systems[21] to the best of our knowledge there are currently no reports describing their reaction with nitrile oxide dipoles, nor are there any reports on solid phase conjugation of oligonucleotides with ring strained alkynes. In the present communication we report the first preparation of a DNA ligated cyclooctyne and demonstrate its utility in strain promoted nitrile oxide click cycloadditions.

The desired DNA–cyclooctyne was constructed by manual solid phase synthesis employing the phosphoramidite building block **4**. Synthesis of this key intermediate, shown in Scheme 1, was achieved in three steps from the dibromobicycle **1**, itself obtained by a known procedure[22] from commercially available *cis*-cycloheptene. Ring opening of **1** with 1,4-butanediol in the presence of silver perchlorate afforded the bromoalkene **2**, which was used further without purification. Sodium hydride induced elimination of HBr furnished the ring strained hydroxyalkyne **3** in 52% yield. Finally, conversion to the phosphoramidite **4** was achieved by reaction with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite in acetonitrile with benzylmercaptotetrazole as activator in the presence of diisopropylamine (Scheme 1).

The cyclooctyne bearing phosphoramidite building block **4** was attached directly to the 5′-position of resin supported-DNA **5a**. Following the standard deprotection-cleavage protocol HPLC analysis of the unpurified DNA **7a** indicated quantitative conversion of **5a** to the CPG-bound DNA–cyclooctyne **6a** (Scheme 2). The structure of the new alkyne **7a** was confirmed by MALDI-TOF-MS. Having established the compatibility of the strained cyclooctyne to the general



Scheme 1

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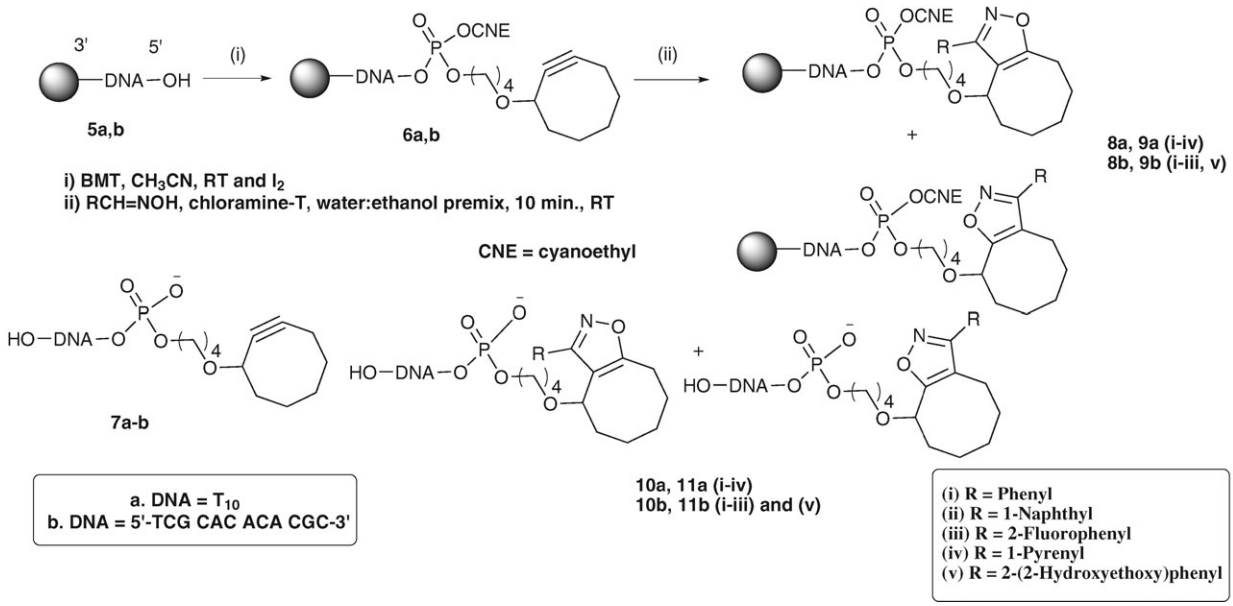

Scheme 2

conditions of DNA synthesis, cleavage and deprotection the DNA–alkyne **7b**, bearing all four natural DNA nucleobases, was prepared by the same reaction sequence. Quantitative coupling was evidenced by HPLC analysis, and MALDI-TOF-MS data confirmed the structural integrity of **7b**.

The reactivity of the resin bound DNA–cycloalkyne **6a** towards nitrile oxide click cycloadditon was tested by exposing it to a premixed solution of benzaldehyde oxime (16 eq.) and chloramine-T (16 eq.) in 50% aqueous ethanol. Progress was monitored by HPLC and reaction found to be complete after just 10 minutes at room temperature (Scheme 2). Following workup, cleavage and deprotection, near-quantitative conversion to the regioisomeric isoxazole–DNA conjugates **10a(i)** and **11a(i)**, was evidenced by HPLC (Fig. 1a,b). Reaction was observed to proceed almost without regiochemical preference and MALDI-TOF-MS confirmed the structural integrity of the new conjugates.

The scope of reaction was tested with a range of *in situ* generated nitrile oxides including, naphthaldehyde 1-nitrile oxide, 2-fluorophenyl 1-nitrile oxide and pyrenyl 1-nitrile oxide prepared from the corresponding oximes upon reaction with chloramine-T. All cycloladditions, bar that with the pyrene substrate were conducted in aqueous ethanol. For pyrenyl 1-nitrile oxide optimal progress was noted in DMF:ethanol. In each case the reaction was terminated after 10 minutes and near quantitative conversion to the click ligated isomeric products **10a(ii–iv)** and **11a(ii–iv)** was confirmed by HPLC analysis following cleavage of the DNA from the resin. As expected, cycloaddition proceeded almost without regard for regioselectivity and the extended retention time of the isomeric products through **10/11a(i)**, **(ii)** and **(iv)** reflects their increasing aromatic character. MALDI-TOF-MS data confirmed the structures of the conjugates **10a/11a(ii–iv)**.

Oligonucleotides bearing reporter groups are useful tools in molecular biology and diagnostics and to prove the general utility of the nitrile oxide–cyclooctyne click methodology attachment of a coumarin label has been demonstrated. Coumarin 6-carboxaldehyde is commercially available and the corresponding oxime **12** was readily prepared by the standard method. To facilitate solubilization of the reacting

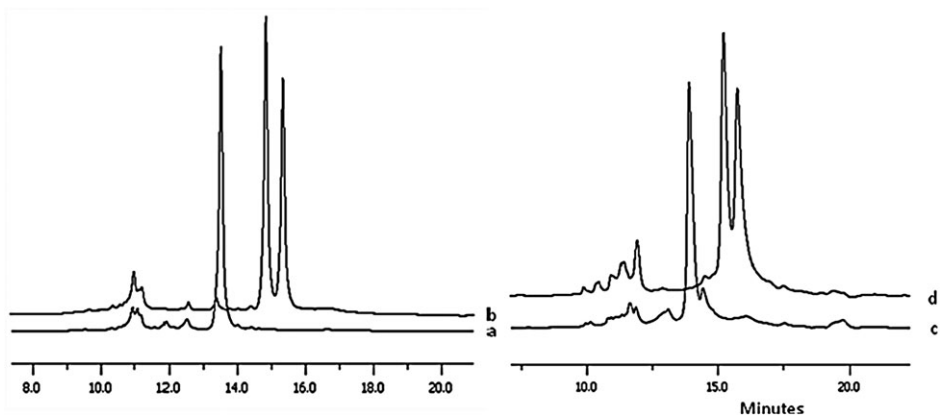

Fig. 1 Reversed-phase HPLC analysis of crude reaction products (UV absorbance at 260 nm *vs.* time) (a) DNA–cyclooctyne (**7a**), (b) isoxazole–DNA **10a (i)/11a(i)**, (c) DNA–cyclooctyne (**7b**), (d) isoxazole–DNA **10b(iii)/11b(iii)**.

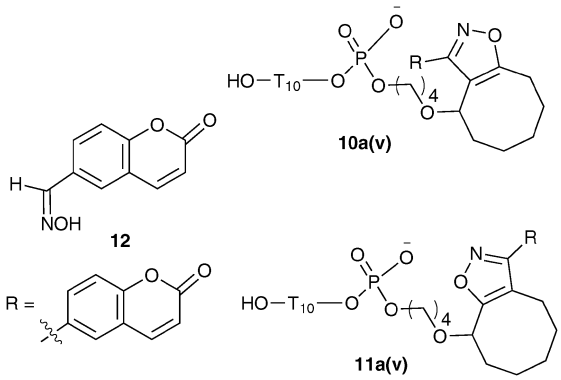

Fig. 2 Structure of the coumarin oxime **12** and the click ligated products.

partners, both the reaction to generate the nitrile oxide and the cycloaddition step were conducted in a mixture of DMF and EtOH. In this solvent mixture conjugation was complete within 10 minutes at room temperature. Regioisomeric cycloaddition products **10/11a(v)**, Fig. 2, were formed in almost equal amounts.

Having verified the potential of resin supported decathymidylate–cyclooctyne **5a** as a click partner with nitrile oxide dipoles we turned our attention to the CPG-supported dodecamer **5b**, 5′-TCG CAC ACA CGC-3′. Theoretically the mixed DNA presents a greater challenge to the synthetic chemist. However, HPLC analysis of the raw reaction products obtained after exposure of **6b** to benzonitrile oxide, generated *in situ* from benzaldehyde oxime (16 eq.) and chloramine-T (16 eq.) in water : ethanol (1 : 1), clearly shows, after 10 minutes at room temperature, two new peaks corresponding to the expected regioisomeric cycloadducts **10b(i)** and **11b(i)**. Conversion to the isoxazole conjugated oligonucleotides was nearly quantitative and MALDI-TOF-MS unambiguously confirmed the structure of the ligated DNA products **10b(i)**, **11b(i)**.

To examine the generality of the resin supported DNA–cyclooctyne–nitrile oxide click cycloaddition as an approach to chemically modified oligonucleotides reaction of **6b** was further explored with naphthaldehyde 1-nitrile oxide, 2-fluorophenyl 1-nitrile oxide and 2-hydroxyethoxyphenyl 1-nitrile oxide. Again the dipoles were generated *in situ* from the corresponding oximes using chloramine-T. Quantitative conversion to isomeric click products **10b**/**11b(ii)**, **(iii)**, **(v)** was confirmed by HPLC analysis of the crude reaction products obtained after cleavage and deprotection from the resin, shown for **10b(iii)** in Fig. 1c,d. In all cases the click reaction was completed in 10 minutes at room temperature and MALDI-TOF-MS data confirmed the structures the new conjugates.

In conclusion, to the best of our knowledge we have demonstrated the first example of resin-supported DNA conjugation by strain promoted cyclooctyne–nitrile oxide click cycloaddition. The reaction proceeds without any metal catalyst, in 10 minutes at room temperature. When compared with their acyclic analogues, the reduction in activation barrier associated with use of the cyclic alkyne means that the click reaction could be promoted with a much reduced concentration of dipole. The reaction is tolerant of nitrile oxide partners bearing bulky aryl groups and of those with electron withdrawing groups, it is also compatible with the hydroxy functional group, which could represent a useful handle for further modification of DNA. Finally, reaction with coumarin 6-carboxaldoxime provides proof of concept for the application of this method to the provision of fluorescently labelled conjugates. These results are of interest for a variety of future applications in chemical biology and material science.

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