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Synthesis of new building blocks for use in supramolecular DNA architectures

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New nucleoside building blocks for the synthesis of functional DNA are presented. A porphyrin-bis nucleoside dU-porphyrin-dU was synthesised from a di-acetylene-substituted porphyrin using Sonogashira coupling with 5-iodo deoxy uridine. The same strategy was used to obtain a new terpy-functionalised nucleoside dU^{terpy}. This building block can be metallated with ruthenium(II) either to make a mono-nucleoside ruthenium complex $(dU^{terpy})Ru^{II}$(terpy), or to connect two building blocks to create a bis-nucleoside $(dU^{terpy})_2Ru^{II}$. The terpy nucleoside building block dU^{terpy} was incorporated into short strands of DNA to give TXT, TXXT and TXXXT as sequences (X = dU^{terpy}). The functionalised DNA has the potential to create supramolecular assemblies through metal complexation.

Keywords: porphyrin; terpy metal complex; nucleoside; DNA scaffold; functional DNA

Introduction

The development of synthetic protocols for modified nucleotides and their incorporation into DNA via phosphoramidite chemistry has made it possible to establish the use of oligonucleotides as a supramolecular scaffold for the synthesis of new functional molecules. In this way, substituents such as metal complexes (*1*, *2*), small organic molecules (*3–7*) and chromophores (*8–12*) can be attached to the DNA in a sequence-specific manner and – upon duplex formation – placed in a predetermined, three-dimensional arrangement. The substituents are thus located either in the major or the minor groove of the DNA, and can lead to stacked arrays of chromophores. The substituents can be incorporated into the DNA either in one strand or in complementary strands (*13*) where new supramolecular assemblies are obtained after hybridisation. Also, the substitution of the nucleobase can lead to new supramolecular arrays (*14–16*). The detailed analysis of the thus obtained functional DNA and the exploration of the new tailor-made molecules through sequence-specific incorporation of the modifications are, however, still in its infancy. Nevertheless, given the diversity of the building blocks already available, this will lead the concept of using DNA as a supramolecular scaffold forward (*17–19*), and open the way to a field which may be termed DNA architectonics (Figure 1).

We are exploring the use of DNA as a supramolecular scaffold to create helical porphyrin arrays and established a general synthetic route to porphyrin–DNA (*20–23*). The successful incorporation of different porphyrins, i.e. tetra phenyl porphyrin (TPP) (*24*) and diphenyl porphyrin (DPP) (*25*), shows that the electronic properties of the supramolecular array strongly depend on the structure of the modification, and the interactions can thus be fine-tuned simply by reprogramming the DNA synthesizer. In particular, the attachment of the porphyrins on complementary strands, which leads to a stable zipper array, allows the reversible creation of photonic wires when using different porphyrins (*26*). Other work has also been concerned with the attachment of porphyrins to DNA as CD markers (*27–30*), or for both internal (*31*) (base replacement) and external (*32–34*) modifications of the DNA. Here, we now present the synthesis of new nucleotide building blocks, which have great potential in the design of DNA-based supramolecular functional assemblies. The building blocks are based on either a porphyrin or a terpy metal complex (Figure 2).

Synthesis of the bis-nucleoside-substituted porphyrin

The porphyrin dinucleoside building block **1** was synthesised according to the route outlined in Scheme 1. The porphyrin itself is synthesised from the corresponding dipyrromethane **6** by 2+2 condensation with the protected acetylene-derivatised benzaldehyde **5** (*35*), and obtained as free-base porphyrin after column chromatography. Metallation using zinc acetate in refluxing chloroform–methanol is quantitative, and the metallated porphyrin **7** can be isolated after evaporation of the solvent and filtration with DCM. Deprotection of the acetylene using KOH in refluxing toluene gives the free bis-acetylene porphyrin **8**. According to our previous protocol to connect an acetylene porphyrin to deoxyuridine, the bis-nucleotide was synthesised by

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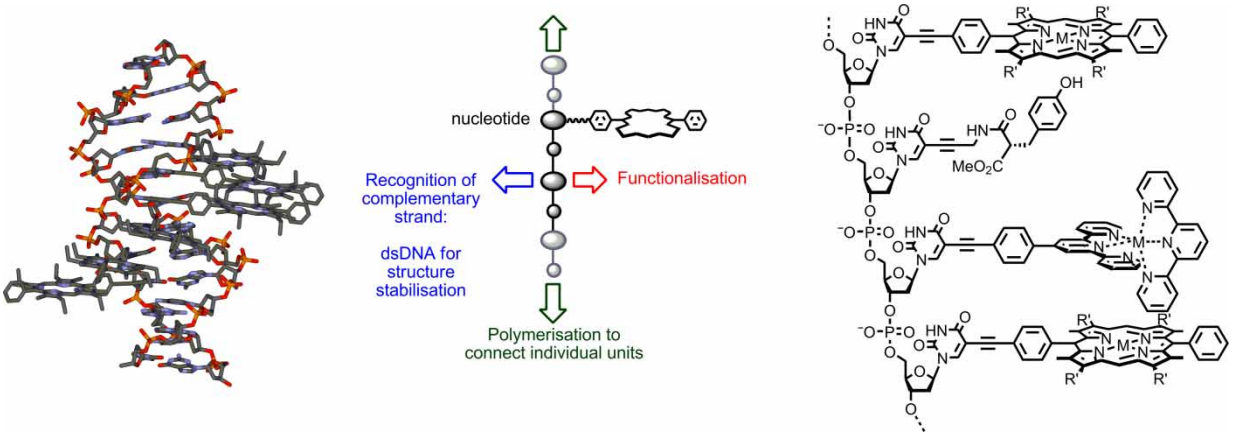


Figure 1. Concept of using DNA as a supramolecular scaffold to create functional molecules (DNA architectonics) with a modelled multiporphyrin array as an example.

Pd–Cu-catalysed Sonogashira cross-coupling with 5-iodo deoxy-uridine **9**. Product **1** was obtained pure after column chromatography and re-crystallisation from DCM–methanol at 4°C in 64% yield. This new compound could well serve as a cross-linking agent in DNA synthesis, or by self-condensation lead to helical-stacked porphyrin arrays, both of which we are currently investigating.

Synthesis of the dU^{terpy} building block

The second new set of building blocks is based on terpyridine as a potential chelating metal ligand. Bipyridyl-derived building blocks have been synthesised previously (*1*, *2*), and the successful incorporation of their ruthenium complexes was reported. The terpy ligand, however, would offer the possibility to form complexes in a linear fashion to form directional supramolecular complexes. Terpy ligands were attached to the sugar moiety of the nucleoside via propargyl amine linkers, but our type of attachment gives a more rigid arrangement, in line with other reports (*38–40*).

The acetylene-substituted terpy (*36*, *37*) was synthesised from commercially available 2-acetyl pyridine **10** and 4-bromobenzaldehyde **11** (Scheme 2) in 45% yield. The acetylene was introduced into **12** via Pd–Cu-catalysed Sonogashira cross-coupling with the protected acetylene 2-methylbut-3-yn-2-ol **13** (21% yield). After deprotection of the acetylene **14** using potassium hydroxide in refluxing toluene (61% yield), the terpy **15** was attached to the dimethoxy-trityl (DMT) protected iodo deoxy uridine **9** by Sonogashira coupling to give dU^{terpy} **2** in 62% yield. All the terpy-containing products can easily be visualised on TLC due to their characteristic blue luminescence upon UV irradiation, or by staining with a saturated ethanolic Fe^{III} solution, giving a brown colour of the iron terpy complex.

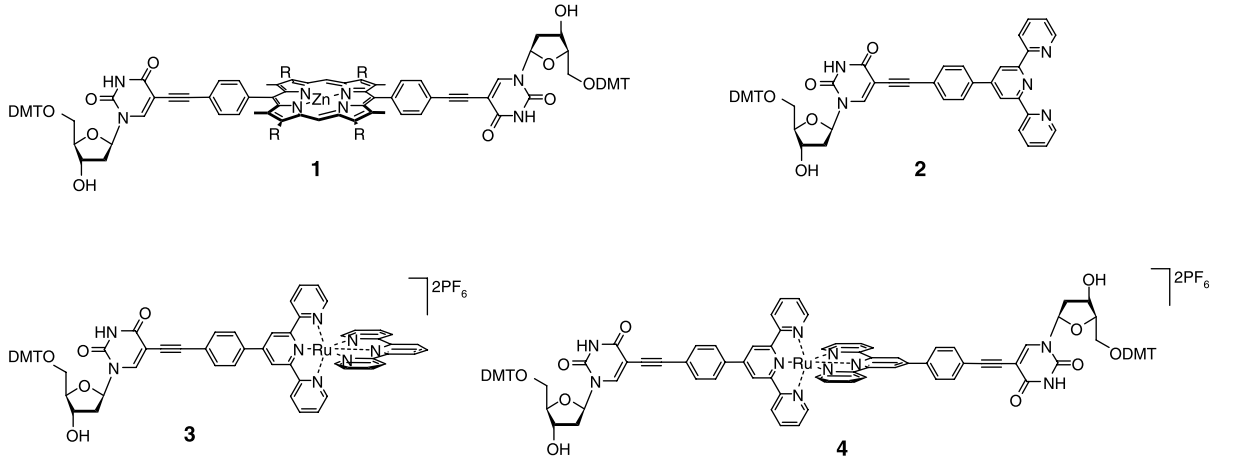
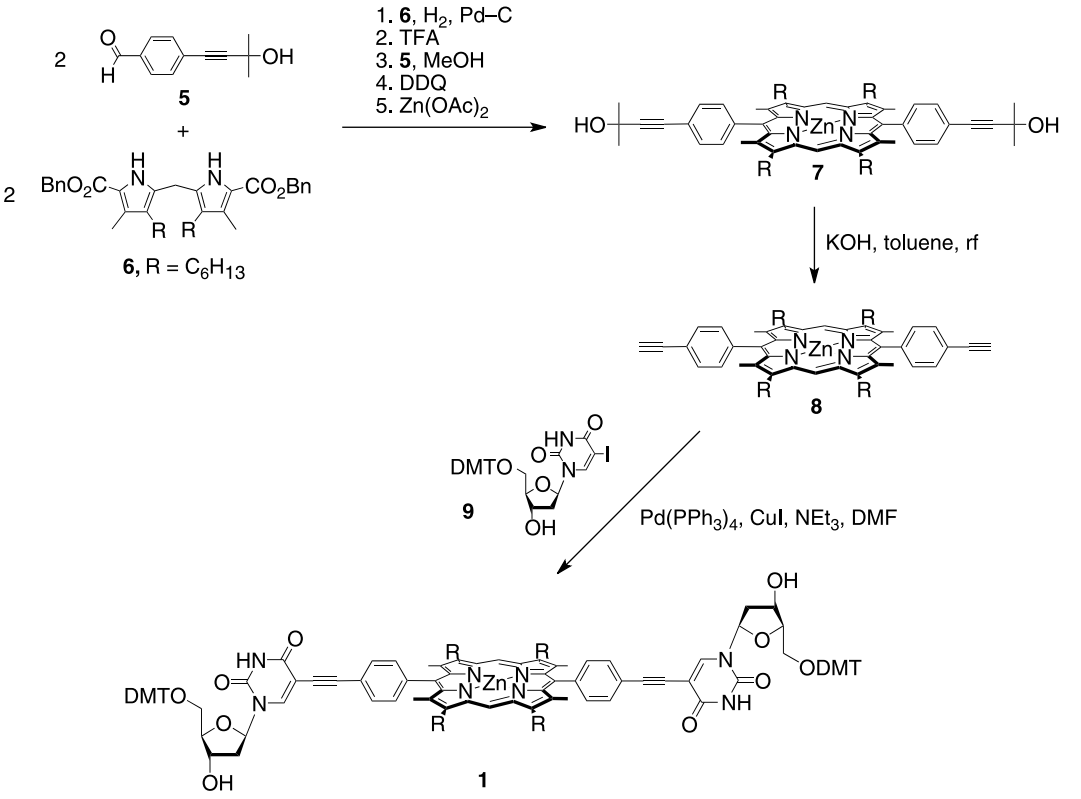
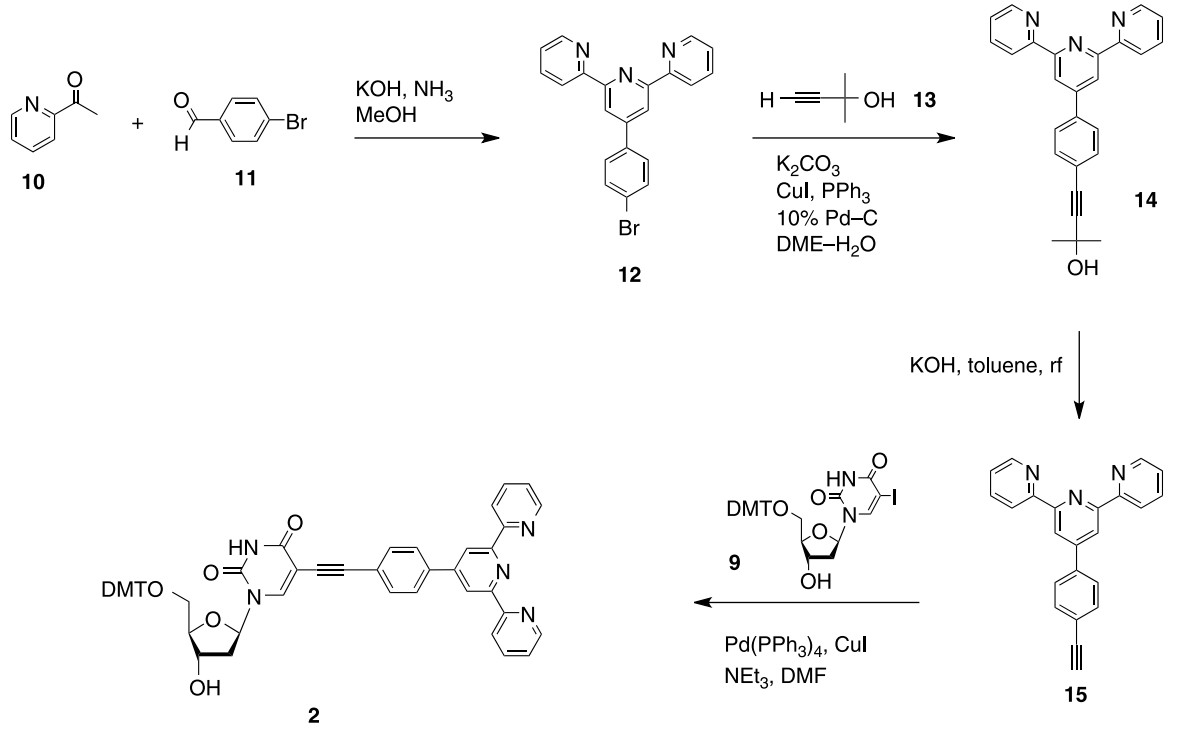


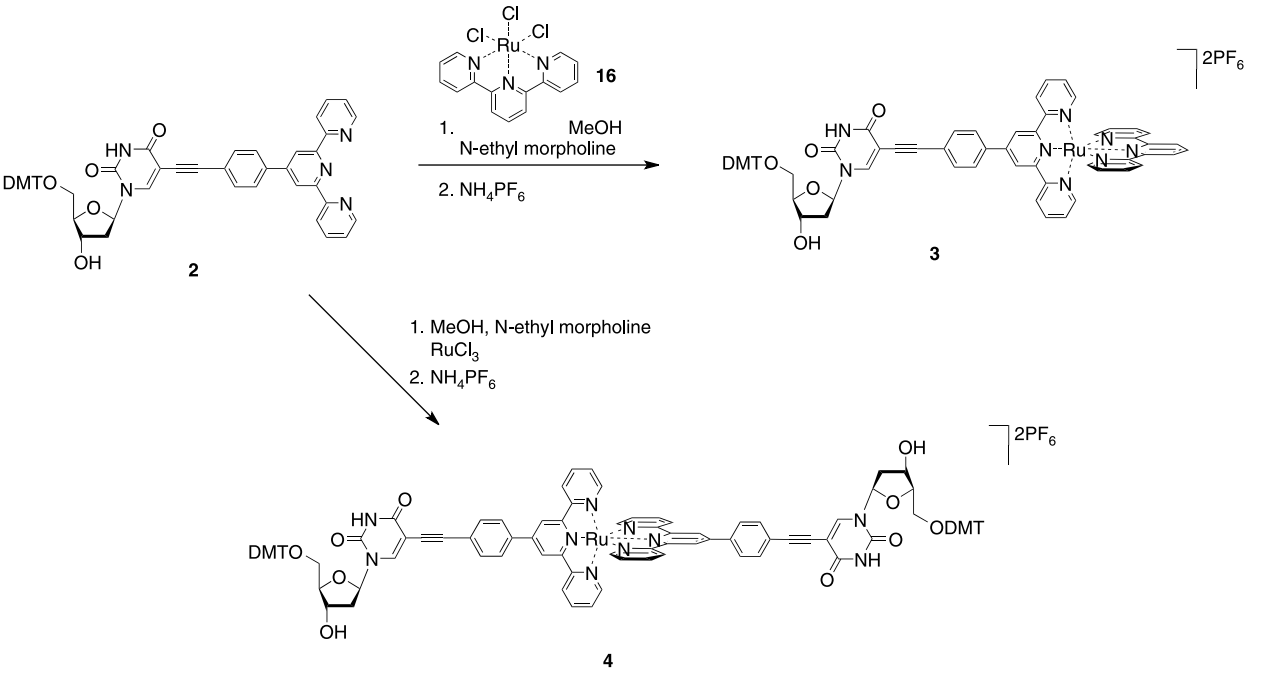
Figure 2. Porphyrin-nucleotide and terpy-nucleotide building blocks.



Scheme 1. Synthesis of **1**.



Scheme 2. Synthesis of the terpy-substituted deoxy-uridine dU^{terpy}.



Scheme 3. Formation of the ruthenium complexes of dU^{terpy}.

Synthesis and properties of ruthenium(II) complexes of dU^{terpy}

Formation of the ruthenium complex with the preformed terpy–ruthenium complex **16** proceeded smoothly from a refluxing methanolic solution (*37*), yielding the ruthenium terpy nucleoside (dU^{terpy})Ru(terpy) **3** (Scheme 3). Initially, the ruthenium complex is isolated as chloride salt, which is then converted to the more soluble PF_6 salt by trituration of an acetonitrile solution with aqueous ammonium hexafluoro phosphate. The product was isolated in overall 52% yield. Alternatively, the terpy nucleoside can be dimerised with ruthenium trichloride in refluxing methanol, thus giving access to the dinucleoside building block $(dU^{terpy})_2$-Ru **4**. As with **3**, the complex was initially formed as the chloride salt and subsequently converted into the PF_6 salt, and the overall yield for this synthesis was 31%.

The UV–vis spectra of the terpy nucleosides measured in acetonitrile are shown in Figure 3. The building block **2** shows two absorption maxima at 284 nm ($\log \varepsilon = 4.65$) and 324 nm ($\log \varepsilon = 4.64$), corresponding to the nucleobase and the terpy moiety. The absorbance of the nucleobase shows a bathochromic shift of 24 nm, indicating some electronic perturbation due to the attached aromatic terpy system. This was so far not observed with other substituents such as porphyrins. The ruthenium complex **3** shows absorbances at 230 nm ($\log \varepsilon = 5.07$), 273 nm ($\log \varepsilon = 5.09$), 308 nm ($\log \varepsilon = 5.15$) and 486 nm ($\log \varepsilon = 4.71$).

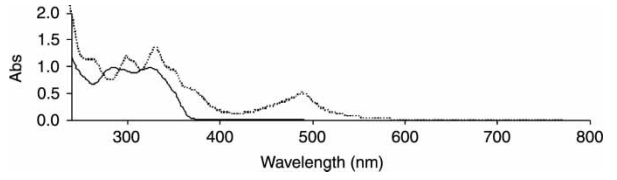
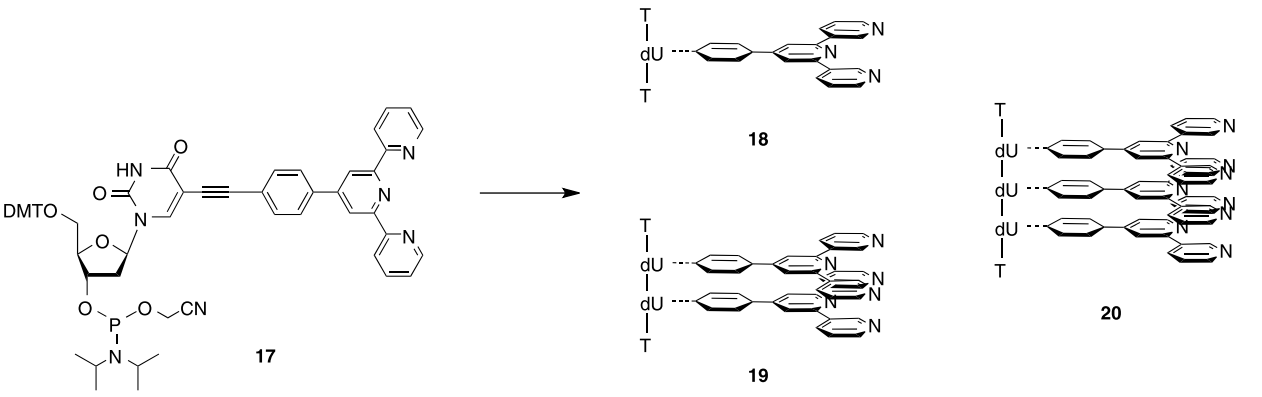


Figure 3. UV–vis spectra of dU^{terpy} **2** (solid line) and of (dU^{terpy})Ru(terpy) **3** (dashed line) ($c = 2.22 \times 10^{-5}$ M in acetonitrile).

Both ruthenium-containing building blocks **3** and **4** showed decomposition upon recrystallisation from acetonitrile–toluene (2:1), but not from acetonitrile–water (2:1). Both NMR and MS analyses of the obtained crystalline products revealed loss of the DMT-protecting group, which normally occurs under acidic conditions. In the case of **4**, further decomposition to the deprotected dU^{terpy} was observed. Analysis of the supernatant showed the formation of benzoic acid, which was detected by its typical fragmentation pattern in the mass spectrum and was not present before treatment of the solution with dU^{terpy}. We therefore do not rule out the possibility that the dU^{terpy} building blocks can catalyse the oxidation of toluene to benzoic acid in the presence of oxygen.

DNA synthesis with dU^{terpy}

Initial studies with the terpy nucleoside for DNA synthesis show that building block **2** can be incorporated into DNA (Scheme 4). The formation of the phosphoramidite **17** was achieved according to standard phosphitylation methods using CEP-Cl as the reagent in dry DCM. The phosphoramidite was then used directly in a DNA



Scheme 4. Solid-phase synthesis of terpy-containing DNA strands using standard phosphoramidite chemistry.

synthesizer for the synthesis of the short oligonucleotide TXXXT (X = dU^{terpy}). Cleavage of the strands from the solid supports using concentrated ammonia overnight and subsequent preliminary purification using NAP-5 and poly-pack columns gave a mixture of the strands TXT **18**, TXXT **19** and TXXXT **20**, as confirmed by MALDI-ToF and UV–vis spectroscopy. The presence of high amounts of the shorter sequences with only one or two modifications indicates that the capping step in the synthesis after introduction of the modification is not as efficient as anticipated. A control synthesis without capping gave the same mixture of strands. In addition, even though four coupling cycles were run with **17**, only three insertions of the terpy building block were observed. The strands were also subjected to denaturing PAGE, but the excised segments containing the terpy DNA which appeared as a streaky band again showed mixtures of the three DNA strands. Separation could so far not be achieved satisfactorily due to the large streaking of the terpy DNA in both PAGE and HPLC. The synthesis and purification of these poly-terpy DNA strands need to be optimised, but the preliminary experiments show that new supramolecular architectures can eventually be obtained incorporating terpy metal ligands for further complexation with various metals.

Summary

In conclusion, we have synthesised new building blocks which can be used in automated DNA synthesis to create new supramolecular architectures. The porphyrin-bis nucleoside could be used to cross-link DNA strands. Formation of helical-stacked porphyrin arrays could be achieved by self-condensation of the corresponding bis-phosphoramidite, which would be more rigid than the corresponding porphyrin stacks that are obtained from mono-substituted nucleosides. The terpy-substituted nucleoside could be incorporated into DNA strands, but both synthesis and purification protocols will have to be optimised. The terpy unit can be used to form complexes with ruthenium(II), though a variety of other metals could be used as well. The exploration of the potential of all building blocks is currently under way.

Acknowledgements

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