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A series of strained alkyynes were prepared from 2,2'-dihydroxybiaryls. Several were characterised by X-ray crystallography, revealing strained C(sp)-C(sp)-C(sp³) bond angles in the range of 163-167°. Their cycloadditions with azides proceed without a catalyst. Functionalised versions of these reagents have potential applications to materials synthesis and bioconjugations.

Azides react with terminal alkyynes to form a triazole in what is commonly referred to as a ‘click’ reaction. Reactions of this type proceed under mild conditions, in high conversion and without the requirement for a copper catalyst. The undesirable requirement for the use of a copper catalyst can be eliminated through the use of reactive strained alkyynes in ‘Strain-Activated Alkyne Azide Cycloaddition’. Examples of recently-reported strained alkyynes are illustrated in Figure 1.

Figure 1. Examples of strained alkyynes which react with azides without the requirement for a copper catalyst. R represents a point of attachment to either a biomolecule or other functional group.

The majority of reported strained alkyynes contain 8-membered rings, however there are some examples of 9-membered ring reagents such as the 2-amidobenzene sulphonamide cyclononane and even a 19-membered crown ether, both illustrated in Figure 1. Thiacycloalkyne and dibenzoselencycloheptynes have also been reported.

Strained alkyynes have also been used to conjugate functional polymers, for example to combine contrasting chains and to form crosslinks in the formation of hydrogels. The undesirable requirement for the use of a copper catalyst can be eliminated through the use of reactive strained alkyynes in ‘Strain-Activated Alkyne Azide Cycloaddition’. Examples of recently-reported strained alkyynes are illustrated in Figure 1.

Following a single precedent in the literature, we investigated the reaction of biaryl diols with 1,4-ditosyl-but-2-ynyl, which resulted in the formation of cyclic alkyne 1-4 (Figure 2). A literature search revealed just two other reports of this heterocyclic structure, both binaphthyl-derived, whilst their reactions with azides have not been reported. The 2,2'-biphenols and their strained-alkyne derivatives are racemic, however compound 2 was prepared as a single enantiomer derived from (R)-BINOL. Compounds 2-4 were characterised by X-ray crystallography (Figure 3) which served to confirm the strained nature of the triple bonds in each product. The sp angles at the triple bonds (C(sp)-C(sp)-C(sp³)) in 3 and 4 were 166-167°, however, due to the presence of three crystallographically independent molecules in the unit cell of 2, the sp bond angles in this molecule ranged from 166.9-163.0° with an average of ca 165°. The high aryl/aryl torsion angles (Figure 3) reflect the wide spacing required to accommodate the linear butyne component in the ring. This angle increased to 112.5° in the dibromo 3 and 122.1° in the tetrabromo compound 4.
The strain in the alkyne is reflected in the reactivity of 1-4 towards benzyl azide and arylazides (Scheme 1), which resulted in formation of cycloaddition products 5-8 in high yields, at room temperature, and without the need for a metal catalyst. The X-ray crystallographic structure of cycloadduct 5a (Figure 4) revealed a low torsion angle between the aromatic rings of just 43°, reflecting the greater flexibility of the triazolyldimethylene bridge.

**Scheme 1.** Cycloadditions of strained alkynes with benzyl azide, aryl azides and a nitrile oxide.

Conversions to cycloadducts were typically ca. 90% or higher at 40 °C within 24 h in several cases, however the reactions could be run for several days without substrate decomposition, to generate products in high yield even at the lower temperature. The cycloaddition of a nitrile oxide (generated in situ from the chloride precursor)23 to give adduct 9, was also completed (Scheme 1).

**Table 1.** Summary of second-order rate constants for cycloaddition reactions.

| Produ ct | Temp / °C | Solvent | [alkyne] / M | Rate constant |
|--------|-----------|---------|-------------|---------------|
| 5a     | 25        | MeCN    | 0.25        | 1.85 x 10^-4 M^-1s^-1 |
| 5a     | 40        | MeCN    | 0.25        | 7.56 x 10^-4 M^-1s^-1 |
| 5a     | 60        | MeCN    | 0.25        | 3.12 x 10^-4 M^-1s^-1 |
| 5a     | 25        | CDCl3   | 0.10        | 1.28 x 10^-4 M^-1s^-1 |
| 5b     | 25        | CDCl3   | 0.10        | 1.41 x 10^-4 M^-1s^-1 |
| 5c     | 25        | CDCl3   | 0.10        | 3.64 x 10^-5 M^-1s^-1 |
| 15     | 25        | MeCN    | 0.25        | 2.50 x 10^-4 M^-1s^-1 |
| 7      | 25        | CDCl3   | 0.10        | 3.07 x 10^-5 M^-1s^-1 |
| 8      | 25        | CDCl3   | 0.10        | 1.24 x 10^-4 M^-1s^-1 |
| 8      | 40        | CDCl3   | 0.10        | 5.00 x 10^-4 M^-1s^-1 |
| 8      | 60        | CDCl3   | 0.10        | 1.69 x 10^-3 M^-1s^-1 |

a. Alkyne:azide = 1:1. b. 81% conv. in 24 h. c. 89% conv. in 12 h. d. Benzyl azide adduct 1; ΔE‡ = 15.9 kcal/mol, ΔH‡ = 15.2 kcal/mol, ΔS‡ = -24.3 cal/mol.K. e. 96% yield in 10 days. f. 92% yield in 24 h. g. p-Tolyldiazide adduct 8; ΔE‡ = 14.6 kcal/mol, ΔH‡ = 14.0 kcal/mol, ΔS‡ = -29.2 cal/mol.K.

The rate constants for 1, within the temperature range examined, are similar to that reported for benzyl azide with 'OCT' (2.4 x 10^-3 M^-1s^-1), but lower than those of other cyclooctyne reagents illustrated in Figure 1 (DIFO; 7. X 10^-2, etc.).
DIBO; 0.17, DIAB; 0.31, BCN; 0.11-0.29, BARAC; 0.96 M⁻¹s⁻¹). The relative reactivities are also illustrated in the distorted sp bond angles of 161/151° (DIF02) and 153° (BARAC).30 However the reactivity of 1 and its derivatives is sufficient for it to be a practical reagent for use in synthetic chemistry applications under mild, Cu-free conditions.

Whilst following the cycloaddition of 1 with benzyl azide at 60 °C, we observed that cycloadduct 5a exhibited significant peak broadening in the ¹H-NMR spectrum whilst the starting material 1 did not. A systematic study of their variable temperature ¹H-NMR spectra, in d⁵-DMSO was completed and this indicated that 1 did not undergo helical inversion even up to 100 °C, whereas 5a exhibited coalescence of the OCH₂ peaks at ca. 56 °C. The ΔG# required for atropisomer interconversion was calculated to be 16.3 kcal mol⁻¹ (Supporting Information). The related aliphatic compound 10 was independently synthesised13 and its OCH₂ peak signals were found to broaden and coalesce at ca. 74 °C (Figure 5; biaryl angle 55.6°, ΔG# required for atropisomer interconversion ca. 16.7 kcal mol⁻¹). For 5a in CD₃CN solvent, coalescence was at a similar temperature of ca. 60 °C. This confirms a much higher level of rigidity in 1 (and hence 2-4) compared to their more flexible cycloadducts and to compound 10 (Figure 5, Supporting Information). Hence the well-defined structures of the cyclic strained alkynes offers the potential for them to be used as the basis of asymmetric ligands, and possibly as building blocks for extended materials.

![Figure 5. Restricted rotation of strained alkyne 1, adduct 5a and aliphatic-ring compound 10. Also shown is the X-ray crystallographic structure of 10.](image)

There is potential for the attachment of derivatives of 1 to proteins and functional groups through the introduction of a linking functional group. In order to demonstrate the potential for this, the ester-functionalised alkyne 11 was prepared in one step from the diphenol 12 in an unoptimised 50% yield (some of the [2+2] and [3+3] products were also isolated; see Supporting Information for structures). Hydrolysis of the ester to acid 13 was followed by formation of the activated NHS ester 14 (Scheme 2). Figure 6 illustrates the X-ray crystallographic structures of both 11 and 13, which exhibit very similar biaryl torsion angles and strained sp bond angles to each other and to

1-4. The reaction of 11 with benzyl azide proceeded (Scheme 1) without a catalyst, giving product 15 in 92% yield at room temperature after a reaction period of 4 days. The second order rate constant for the cycloaddition was similar to that for 1 (Table 1), indicating that the introduction of the ester had not significantly diminished the reactivity of the alkyne.

![Scheme 2. Synthesis of activated NHS ester 14 and subsequent reaction with peptides and a protein.](image)

Reduction of 14 with Dibal resulted in formation of alcohol 16, which also has the potential to be converted to a conjugation reagent. Although lower in reactivity than the most active strained alkynes (Figure 1), we sought to obtain preliminary evidence of its potential for bioconjugation. To obtain a proof of principle in this respect, 14 was reacted with the small peptides substance P, vasopressin and bombesin, and the protein myoglobin, the reactions being followed using FTICR mass spectrometry (Scheme 2). The reactions of peptides with the activated ester 14 in water/methanol solution resulted in partial functionalization to give adducts 17 (see Supporting Information). MS/MS studies confirmed that the lysine residue in each peptide had been functionalised. The subsequent reaction with an excess of benzyl azide in each case occurred with >95% conversion to the ‘clicked’ product 18.

Solutions of myoglobin and 14 in methanol/water were prepared, and after standing for one day at rt were analysed by FTICR. Since myoglobin contains multiple lysine residues, the protein was only partly functionalised (ca 10%) in order to avoid formation of a range of functionalised proteins, which would be more difficult to analyse. An excess of benzyl azide in the same solvent was then added and after 7 days the solutions were analysed by FTICR (Figure 7 features an expansion of one charged form at each stage). Significantly, the subsequent...
cycloaddition reaction of the fraction of functiona-lised protein had undergone >95% conversion to the ‘click’ cycloadduct.

Figure 7. The FTICR-MS spectra of one charge state of myoglobin. Top; without 14, middle; following addition of 14, bottom; following addition of PhCH₂N₃.

In conclusion, we have demonstrated that strained alkynes based on diether derivatives of 2,2’-diphenol react with azides without the need for a metal catalyst. The strained alkyne can be attached to a biomolecule or other group, hence demonstrating the potential for the reagents to be used broadly in synthetic reactions where a Cu catalyst must be avoided, and with potential for use in bioconjugation applications. Importantly, the strained alkynes described herein benefit from extreme ease of synthesis, from readily available and inexpensive starting materials.

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