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Synthesis of Endocyclic Cycloalkyne Amino Acids

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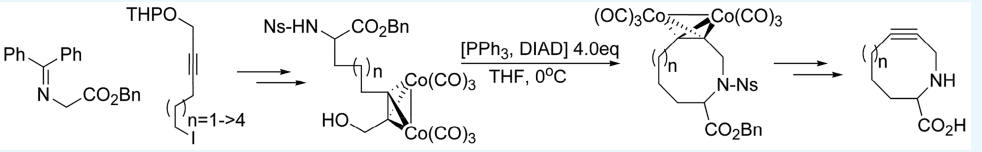

ABSTRACT: "Click-ligation" is a widely adopted and valuable means to ligate biomolecules whereby two appended biologically inert moieties, such as alkynes and azides, link by cycloaddition. For terminal alkynes, Cu^{+1} catalysis is required which degrades oligonucleotides by catalyzing their hydrolysis but is also physiologically incompatible. The smallest activated alkynes that do not require Cu^{+1} catalysis are cyclooctynes or dibenzo-cyclooctynes. For this purpose, there are commercially available nucleosides and amino acids that are appended to these moieties. However, these structures are bulky, dissimilar to native amino acids, and when incorporated within biological molecules could likely perturb native structural configuration. Presented are the syntheses of structural analogues of proline with an inserted propargyl moiety within a series of ring sizes. Moreover, a synthetic pathway to medium-size ring heterocycloalkynes mediated by using mild Mitsunobu conditions in tandem with a Nicholas-related strategy for cyclization is introduced. Avoiding the usual harsh acidic conditions for the Nicholas reaction allows improved functional group compatibility.

INTRODUCTION

Strategies that offer bioorthogonal characteristics for attaching molecules within the cellular environment have become important tools for the study of physiological phenomena. Necessary features of such bioorthogonal reactions include selectivity over being susceptible to reacting with other functional groups occurring within physiological systems.[1] A variety of reaction types fit these requirements such as tetrazine and Staudinger ligations,[2] esterifications, 1,3-dipolar cycloaddition of azides,[3] nitrones,[4] nitrile imines,[5] tetrazines,[6] or diazo derivatives,[7] and hydrazone or oxime formation.[8] One of the most widely used bioorthogonal reactions is the Huisgen 1,3-dipolar cycloaddition employing two appended biologically inert moieties, an alkyne and an azide. As this reaction requires Cu^{+1} catalysis, which is cytotoxic, utilization of a variety of strained cycloalkyne derivatives have been employed. In 2004, Bertozzi[3] and colleagues reported vanguard work regarding the catalyst-free Huisgen reaction of cyclooctyne derivatives with azides and successfully applied it as a bioorthogonal bioimaging method.[9] Many examples of successful strained cycloalkynes are well-characterized in practice and also commercially available.[22]

The premise for development of propargylic heteroatom cycloalkynes is that the combination of reactant destabilization and transition state stabilization can lead toward the design of more reactive cycloalkynes that are paradoxically less strained. Difluoro cyclooctyne is a good example of this, for which alkyne π^* is delocalized via $\sigma^*_{C\text{-}X}$ thus stabilizing its transition state.[23] Stereoelectronically, the incorporation of endocyclic heteroatoms maximizes σ-acceptor hyperconjugation offering superior stabilization due to its antiperiplanar orientation with the alkyne π^*.

Despite the successes of these cycloalkynes, challenges still remain regarding stability, synthesis, and chemoselectivity. A variety of medium-sized strained cycloalkynes have since been synthesized. However, some of these are not readily amenable to chemical biological experiments because of their instability.[10] Therefore, imparting a suitable reactivity to the strained alkynes is important not only for stability and reactivity, but also for orthogonality in chemical biology.

Syntheses of cycloalkynes with endocyclic propargylic heteroatoms (O,N,S) are often achieved by ring closure through harsh Lewis acid-mediated Nicholas strategy. Release of the dicobalt−carbonyl complexed protecting groups leads to the strained cycloalkyne. The first published *N*-heteroatom mediated Nicholas cyclizations succeeded with a tosyl sulfonamide and a propargylic methyl ether[15] using tetrafluoroboric acid diethyl etherate (Figure 1).

Tomooka et al.[11,12] and Kaneda et al.[13] have synthesized medium sized 8, 9, 10, and 11-membered ring cycloalkynes with diendocyclic propargyl O,N,S heteroatom functionalities by a double Nicholas approach (Figure 2). Adjusting reactivity was conferred by altering ring size and heteroatoms based upon results of computational modeling.[14] Most all of these derivatives are stable except for the cyclooctyne derivative

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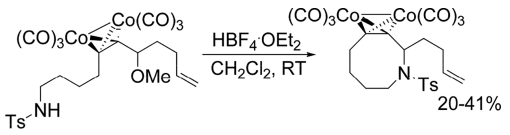


Figure 1. First report of *N*-nucleophilic annulation by acid-mediated Nicholas reaction. Figure adapted from ref 15. Copyright 2009 American Chemical Society.

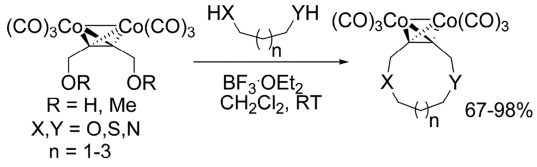


Figure 2. First report of a route for syntheses of double heteroatom cycloalkynes by double Nicholas substitution. Figure reproduced with permission from ref 12. Copyright 2015 Wiley-VCH.

where decomplexation of the dicobalt-CO under oxidative conditions occurs at lower yields. In general, the Lewis acid conditions for ring closure for these types of cycloalkynes preclude derivatization with more sensitive functional moieties. Proposed here is a milder scheme to effect cyclization for synthesis of *N*-propargylic cycloalkynes. In this manner a wider range of functionalization for these molecules is accessible.

This strategy offers a route to *N*-propargylic cycloalkynes by cyclization through a milder Nicholas reaction that is mediated using Mitsunobu conditions to activate an alcohol for nucleophilic displacement. For these cases cyclization is mediated through the dicobalt-CO protected propargyl alcohol and *N*-nosyl moieties of the starting structure. An ester is retained from the starting material as a sentinel functional group through to its final hydrolysis achieving the cycloalkyne amino acid final product. To date no cycloalkyne endocyclic amino acids have yet been reported. Nonetheless, there is extensive interest in cyclic amino acids,[25,26] such as stereocontrol in syntheses of prolines,[27] quaternary α-amino acids,[28] and cyclic triproline peptides.[29]

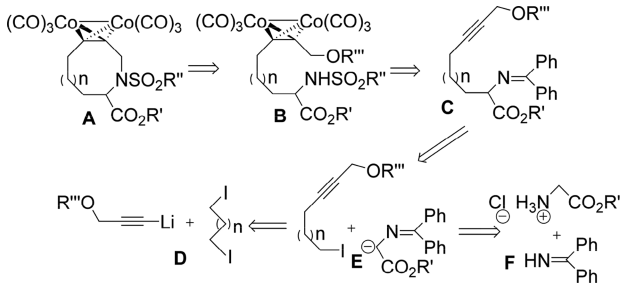
There are many commercially available amino acids that incorporate cyclooctynes and other dienophiles as adducts[16] (Figure 3).

However, these structures are very dissimilar to native amino acids as the strained reactive components are merely appended onto their structure. As an improvement, a strained cycloalkyne component can be likely incorporated internally within an amino acid more closely resembling a proline analogue. To gauge structural perturbation incurred by such a substitution, incorporation within one position of one strand of a well-described triplex collagen system[30] would offer a sensitive evaluation.

RESULTS

As in these prior Lewis acid-mediated Nicholas cyclization strategies (Figures 1,2), the ring closure occurs at the propargylic carbon through release of an ether via an *N*-heteroatom incorporated as a sulfonamide. In this manner a suitably protected amino acid can be considered as a precursor where its side chain incorporating the alkyne component could be retained at the α-carbon position. A retrosynthesis is arranged in Scheme 1 where such an amino ester is configured as the

Scheme 1. Retrosynthetic Pathway for an Amino Acid Incorporated Cycloalkyne for Nicholas Cyclization



Schiff base (**F** → **E**) as a means for addition of the alkyne side chain by its α-alkylation (**E** → **C**) in preparation (**C** → **B**) for Nicholas cyclization (**B** → **A**).

For this objective, the starting material **6a** for this cyclization (Scheme 2) was created through alkylation of the Schiff base of the glycine ester **1** with iodopropyl propargyl methyl ether **2**. This was followed by deprotection of the imine followed by its sulfonylation and alkyne coordination with dicobalt carbonyl.

Benzophenone imine[17] is reacted with amino acid ester hydrochloride to afford the Schiff base **1** under similar conditions to that used by O'Donnell.[18] The latter component **2** is created by alkylation of the acetylide of the propargyl methyl ether, made by its addition to nBuLi/THF at −40 °C, warming to RT, adding 1.5-fold excess of 1,3 diiodopropane in THF stirring at −40 °C, and then refluxing overnight. This was based upon a similar procedure using an α-iodo ω-bromo/chloro alkane in which the terminal ω-halogen was exchanged for the iodide with NaI.[24] However, using a moderate excess of the terminal diodo alkane proves more cost and labor efficient. Alkylation of Schiff base **1** with iodopropylpropargyl methyl ether **2** was initially attempted using O'Donnell conditions with strong base and a phase transfer agent;[18] however, yields were low partially due to ester saponification. This α-alkylation was best effected by adding the Schiff base to KOtBu in THF at 0 °C followed by fast addition of the alkylator **2** at −78 °C and letting warm to RT overnight yielding the α-substituted Schiff base **3**. The imine was hydrolyzed by phase transferred aqueous HCl yielding the amine **4** which was then initially sulfonylated with nosyl chloride in dichloromethane/triethylamine. The disul-

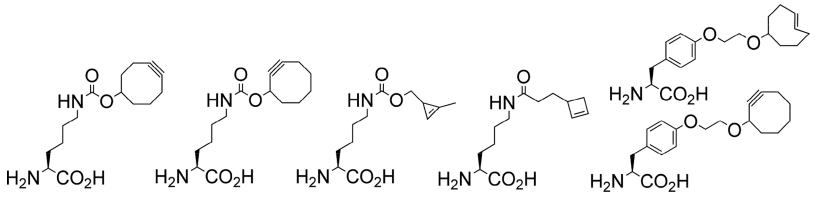


Figure 3. Some available unnatural amino acids with dienophile side chains suitable for [2 + 3] cycloaddition "Click Chemistry".

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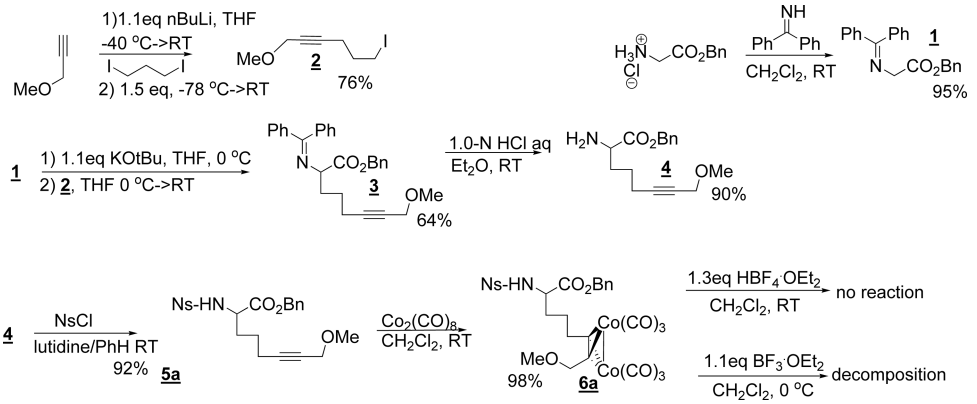
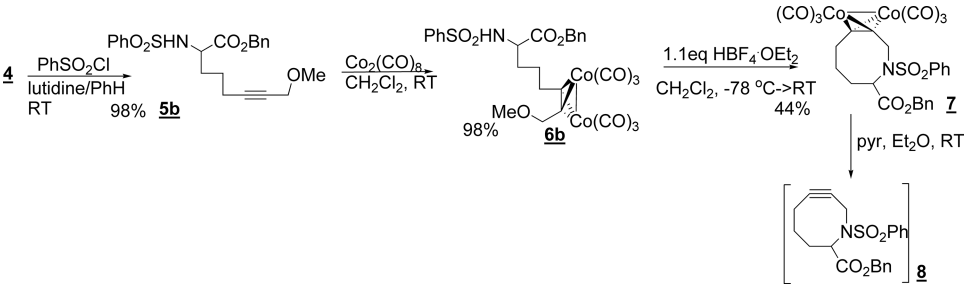
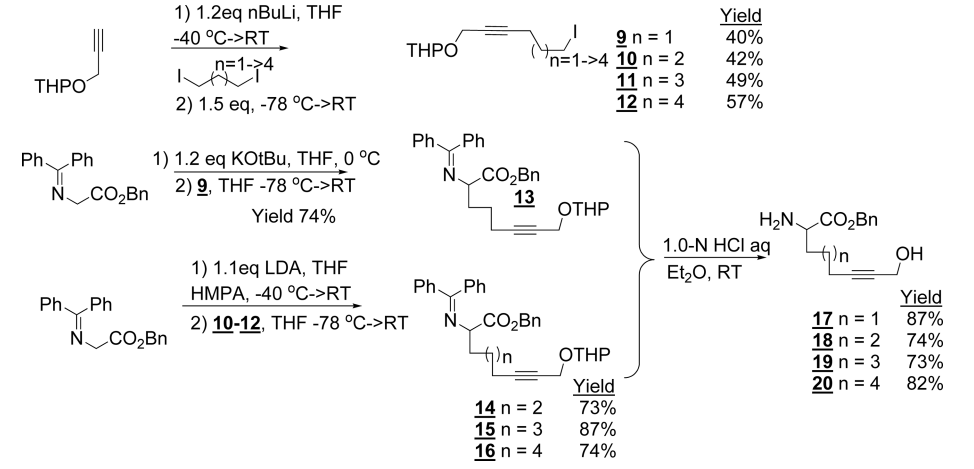
Scheme 2. Attempt for Synthesis of an Endocyclic Cyclooctyne Amino Acid by Acid-Mediated Nicholas Annulations via an *N*-Nosyl Methoxypropargyl Dicobalt Alkyne Adduct



Scheme 3. Successful Acid-Mediated Nicholas Cyclization with the More Nucleophilic Benzene Sulfonamide



Scheme 4. Scheme for Syntheses of Cycloalkyne 9−11 Ring Precursors for Their Annulation Leading to Endocyclic Cycloalkyne Amino Acids



fonylated adduct appeared preferred over the monosulfonylated product. It was hypothesized that the monosubstituted product could position the aryl sulfonyl chloride by π-stacking holding it in proximity. Changing the solvent to benzene and base to 2,6 lutidine on the premise to saturate π-interactions of the reagent eliminated the prevalence of the disubstituted product in favor of the mono- substituted product **5a**. The change of base to lutidine alone was not successful, and use of toluene as solvent was not as effective. The dicobalt adduct **6a** was achieved by adding $Co_2(CO)_8$ to the alkyne in dichloromethane.

It is also important to note at this juncture that initial attempts had been undertaken to cyclize the nosyl amino ester dicobalt-protected propyl propargyl methyl ether via Nicholas reaction[16,19] with $HBF_4{\cdot}OEt_2$ or $BF_3{\cdot}OEt_2$ in dichloromethane yielding no reaction by the former and decomposition products by the latter (Scheme 2). It was surmised that the nosyl moiety imparts insufficient *N*-sulfonamide electron density. In response, the benzenesulfonamido-ester **5b** (sulfonylating with benzenesulfonyl-Cl instead of nosyl-Cl) was synthesized and coordinated to the dicobalt compound **6b** which was cyclized to the dicobalt-coordinated cyclooctyne **7** at 44% yield by its slow addition into a solution of 1.1 equiv of $HBF_4{\cdot}OEt_2$ in dichloromethane under argon (Scheme 3).

Deprotection was beset with difficulty. Removal of the coordinated dicobalt protecting group with ferric nitrate to yield cyclooctyne **8** was problematic with limited product detected and mainly dimer and trimer identified by mass spectroscopy. Better yields were achieved using ceric ammonium nitrate

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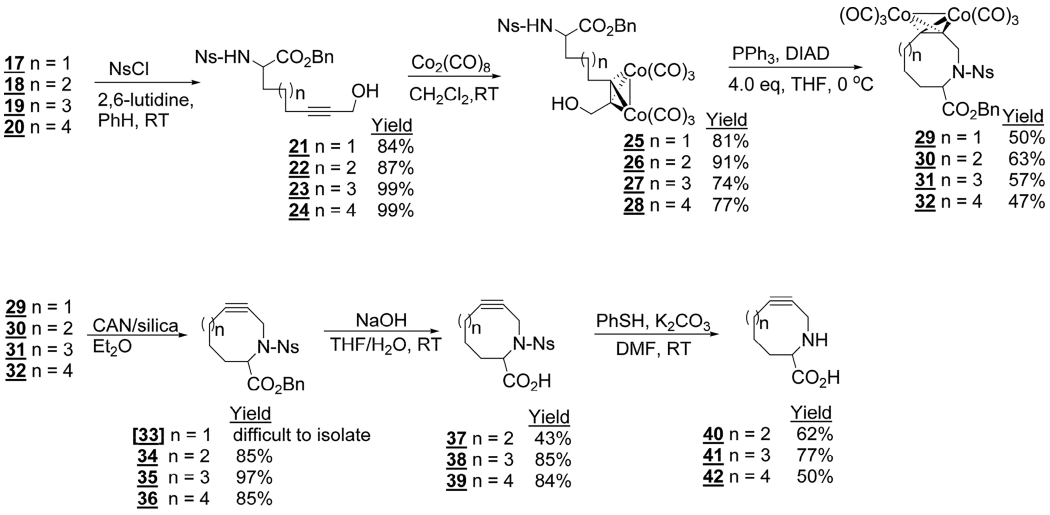
Scheme 5. Scheme for Mitsunobu Annulations Leading to Endocyclic Cycloalkyne 9-11-Ring Amino Acids



(CAN) or pyridine/Et_2O; however, isolation of **8** was fraught with difficulty given its apparent instability at room temperatures upon concentration and exposure to silica gel. Carrying forward without purification, deprotection of the benzenesulfonamide with a variety of conditions gave poor results due to the rather insufficiently selective means available, for example, $LiAlH_4$ at −78 °C or Mg/MeOH.

At this juncture, it was necessary to consider the mildest tenable reaction conditions that are compatible with the strain-relieving alkyne-dicobalt carbonyl adduct cyclization strategy and incorporating an easily deprotectable amine equivalent such as nosyl. Exploiting the milder Mitsunobu reaction conditions was considered because of its broader regio-compatibility. Hence, aside from retaining a compatibly nucleophilic and more easily deprotectable nosyl moiety as per Fukuyama strategy,[20] the only necessary modification was to replace the methyl propargyl ether with a tetrahydropyranyl propargyl ether allowing concomitant deprotection to the alcohol during acid hydrolysis of the Schiff base imine deprotection to the amine. To illustrate its applicability for closure of an array of medium-size rings which are of widespread interest, preparation of 8−11 size ring cycloalkynes was proposed.

For this objective (Scheme 4), iodoalkylpropargyl tetrahydropyranyl ethers **9**−**12** were required and prepared under the same conditions as for preparation of **2**. Preparation of **13** by alkylation of the Schiff base with the iodopropylpropargyl tetrahydropyranyl ether **9** required the same conditions as for the α-substituted Schiff base **3**. However, alkylation with the longer iodoalkyl chain propargyl ethers **10**−**12** under these conditions to create adducts **14**−**16** yielded a side product consistent with β-elimination. This is likely due to the β-hydrogen to the iodide becoming more accessible as the alkyl chain lengthens away from the relatively sterically imposing tetrahydropyranyl ether moiety. Some improvement was had using LDA/THF, however addition of HMPA to increase polar conditions to favor substitution over elimination significantly increased the yield for adducts **14**, **15**, and **16**.

Deprotection of Schiff base (Scheme 4) and tetrahydropyranyl ether was similarly achieved with phase transferred aqueous HCl to create the amino esters **17**−**20**, and subsequent *N*-nosylation to create the NH-nosyl esters **21**−**24** (Scheme 5) was effected with nosyl-Cl in lutidine/benzene. The dicobalt adducts **25**−**28** were created by adding $Co_2(CO)_8$ under argon to the alkynes **21**−**24** in dichloromethane, respectively. Cyclization was effected via a Mitsunobu mechanism with strain relieved through bridging the alkyne with a dicobalt-CO protecting group. Moreover, the electron density-withdrawal from the propargylic carbon by the dicobalt bridge would likely assist this reaction. The dicobalt derivatized starting material **25**−**28** was added slowly dropwise to a suspension of 4 equiv of preformed PPh_3-DIAD zwitterion in THF at 0 °C to generate products **29**−**32** in 47−63% yield. The crystal structure for **29** was obtained showing the expected connectivity and configuration (Figure 6). Careful attention needs to be addressed for using accurate equimolar amounts of both PPh_3 and DIAD to form the zwitterion as either of these reagents in excess will react with the dicobalt-CO bridge. To investigate whether stabilization of the propargylic cation by dicobalt-CO is of utility for the Mitsunobu cyclization, attempted cyclization without this adduct yielded predominantly the cyclohexadecadiyne through dimeric coupling. Deprotection of dicobalt-CO was best achieved with CAN/silica gel in Et_2O[11] to yield the cycloalkynes **34**−**36**. Of the many alkyne-Co decomplexation strategies evaluated, for example, pyr/Et_2O, Et_3NO, CAN/acetone, and $Fe(NO_3)_3$, silica-adsorbed CAN was the most facile and less prone to side products. Unlike the 9−11-ring cycloalkynes **34**−**36**, the cyclooctyne **33** was unstable at room temperature and upon extended exposure to silica gel, making **33** difficult to isolate. Soon after initial purification attempts, mass spectrometry analysis of **33** revealed additional peaks consistent with dimeric and trimeric products, indicative of self-reactions. Conversely, mass spectrometry analysis of **34**−**36** prior to purification showed negligible evidence of respective dimeric and trimeric products. Products **34**−**36** are stable and readily purified. Accordingly, the instability of **33** can be attributed to its increased reactivity because of relatively greater ring-strain.

Regarding **34**−**36** benzyl ester hydrolysis was achieved with NaOH in THF/water yielding the carboxylic acids **37**−**39** of which a crystal structure for **39** was obtained (CIF Supporting Information) consistent with the anticipated structure. Furthermore, nosyl deprotection was effected by PhSH/K_2CO_3 in DMF at room temperature[20] yielding the cycloalkyne amino acids **40**−**42** also in quantitative yield. Final purification was effected by silica gel chromatography eluting with iPrOH/MeOH/NH_3 aq.[21]

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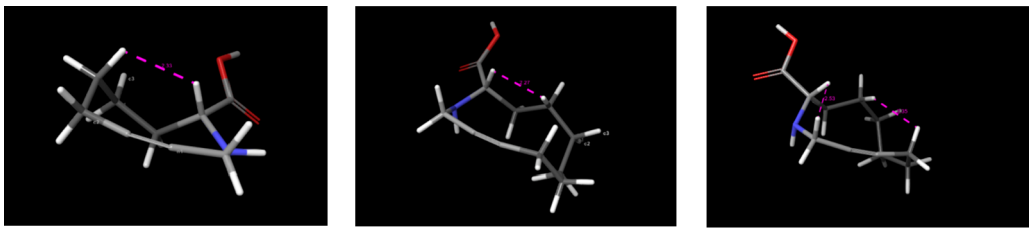

Figure 4A Figure 4B Figure 4C

Figure 4. Molecular structures for (A) cyclononyne **40**, (B) cyclodecyne **41**, and (C) cycloundecyne **42** amino acids were energy minimized; with closest interproton distances calculated for detected NOEs.

Given the asymmetry introduced by incorporation of a single propargylic heteroatom, charge asymmetry of the alkyne carbons would arise. Such an arrangement potentially offers regioselective advantages for cycloadditions. As an indicator of these properties, partial electrostatic potentials (ESP) at the alkyne carbon atoms were calculated for **40**−**42** (Supporting Information, Supplemental Spectra Figure S457).

From NOESY experiments in D_2O, the NOEs detected are also consistent with ring configurations found upon energy minimization using the OPLS3e force field[45] (Figure 4A,B,C). For the cyclononyne amino acid **40**, an NOE is detected between the C1 (α) methine proton and the syn-facial C4 methylene proton of 2.33 Å distance consistent with the energy minimized structure (Figures 4A, 5)). The energy minimized

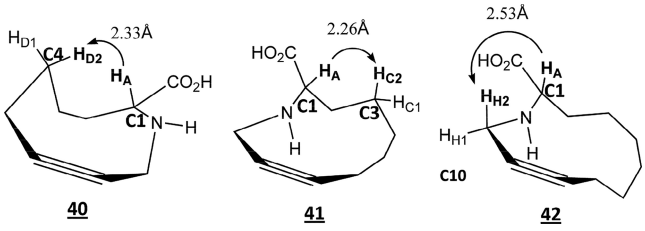

Figure 5. NOEs detected; distances calculated from OPLS3e force field minimized structures.

structure (Figures 4B, 5)) for the cyclodecyne amino acid **41** reveals a 2.26 Å distance between C1 (α) methine proton and the syn-facial C3 methylene proton evident by NOE.

For the cycloundecyne amino acid **42**, an NOE is detected between the C1 (α) methine proton and the syn-facial C10 methylene proton of 2.53 Å distance consistent with the globally minimized structure found upon energy minimization (Figures 4C, 5).

DISCUSSION

A Nicholas cyclization offers a route to strained cycloalkynes by performing ring closure at the propargylic position by coordination of the alkyne π-orbitals conferring configurational accommodation while stabilizing a propargylic carbocation initiated by Lewis acid. A route to medium ring cycloalkynes with single nitrogen heteroatom placed at the propargylic position with an adjacent carboxylate has yet to be reported. During endeavors to synthesize these proline-like analogues, a Mitsunobu-mediated ring closure employing the alkyne-protecting dicobalt-CO which configurationally aids cyclization was developed and employed successfully. This modification offers improved functional group compatibility avoiding the harsh acid conditions required for Nicholas-mediated routes. The aza-cycloalkyne structures were found to be significantly sensitive to dicobalt deprotection conditions. A multitude of conditions were tried, $Fe(NO_3)_3$, CAN/acetone, Et_3NO, Pyr/Et_2O, and TBAF, where application of silica-adsorbed CAN/Et_2O giving near quantitative yield for the 9,10,11-ring aza-cycloalkynes. Unlike for the larger aza-cycloalkynes, stability of the aza-cyclooctyne was very limited, similar to what was reported for the diheteroatom substituted cyclooctynes by Tsunoda.[12] Following ester hydrolysis and nosyl deprotection, the aza-cyclononyne **40** and the larger aza-cycloalkyne amino acids **41** and **42** were found stable for isolation and storage at room temperature. The intent is to introduce an accessible route to amino acid analogues that incorporate strained cycloalkynes with the intention for use in bioorthogonal ligation. The intention of this work is to present milder cyclization conditions via a Mitsunobu alternative to the commonly employed Nicholas/Lewis acid reaction mediated cyclization. In this manner a wider range of functionalization for these molecules are accessible.

EXPERIMENTAL SECTION

Materials and Methods. All chemicals used in syntheses, purification, and comparison analysis were of commercial reagent quality and were used without purification. All reactions involving dry solvents or sensitive agents were performed under argon atmosphere, and glassware dried in a 130 °C drying oven. Dichloromethane, THF, and DMF were dried by standing over 4 Å molecular sieves for a minimum of 48 h. Reactions were monitored by analytical thin-layer chromatography (TLC, Merck silica gel 60 F254 on glass plates). Flash chromatography was performed using Merck silica gel 60 Å (40−63 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded on either 500 or 600 MHz Bruker instruments. Chemical shifts (δ) are reported in ppm relative to residual protonated solvent peaks. ESI mass spectroscopy was implemented with a Waters QTQF instrument.

Synthetic Procedures. Example reactions regarding synthesis of the cyclononyne **40** are described below. Complete Experimental Section is available as Supporting Information.

***14** N-(Diphenylmethylene)-α-([7-[2-tetrahydropyranyloxy]]-6-heptynyl) Glycine, Benzyl Ester.* To an argon-flushed 50 mL flask was added 3 mL of tetrahydrofuran, diisopropylamine (150 μL, 1.1 mmol), chilled to −78 °C. Then *n*-butyllithium (1.6 M hexanes, 750 μL, 1.2 mmol) was added with stirring, and the mixture was let stir for 15 min. The solution was warmed to −40 °C and **1**, benzophenone imine glycine benzyl ester (330 mg, 1.0 mmol) in 5.0 mL of tetrahydrofuran was added over 1 h, and this mixture was let stir for 45 min more. The reaction was rechilled to −78 °C, and then **10**, 1-iodobutyl propargyl tetrahydropyranyl ether (350 mg, 1.1 mmol), was added in 3.0 mL of tetrahydrofuran over 5 min, and then the

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mixture was left to react at room temperature overnight. The reaction was then partitioned between 100 mL of diethyl ether and 100 mL of saturated aqueous ammonium chloride, the organic phase was dried with magnesium sulfate, the solvent was evaporated, and the residue was purified by flash chromatography with 6:1 hexanes/ethyl acetate, 2% triethylamine. yielding 382 mg, 73% yield. [TLC *Rf* = 0.55, 4:1 hexanes/ethyl acetate. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (dd, J_1 = 1.3 Hz, J_2 = 5.3 Hz, 1H), 7.55 (dd, J_1 = 5.3 Hz, J_2 = 1.3 Hz, 1H), 7.41 (t J = 6.1 Hz, 2H), 7.35−7.28 (m, 6H), 7.28−7.15 (m, 6H), 7.05 (m, 1H) 5.12 (d, J = 12.4 Hz, 1H), 5.06 (d, J = 12.4 Hz, 1H), 4.68 (m, 1H), 4.18−4.14 (m, 1H), 4.10−4.05 (m, 2H), 4.01 (t, J = 6.4 Hz, 1H), 3.76−3.71 (m, 1H), 3.44−3.41 (m, 1H) 2.09 (t broad, J = 2.1 Hz, 2H), 1.90−1.84 (m, 2H), 1.77−1.70 (m, 1H), 1.66−1.59 (m, 1H), 1.55−1.47 (m, 2H), 1.47−1.40 (m, 2H), 1.40−1.26 (m, 2H), 1.25−1.18 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.8, 172.1, 170.7, 137.6, 136.6, 135.9, 132.5, 130.6, 130.1, 129.3, 129.1, 128.8, 128.7, 128.5, 128.4, 128.2, 128.1, 127.8, 96.7, 86.3, 75.9, 66.6, 65.3, 62.0, 60.4, 54.6, 33.1, 30.3, 29.7, 28.3, 25.5, 21.1, 19.1, 18.7. m/z $[M+H]^+$ $C_{34}H_{37}NO_4H^+$ calcd MW, 524.2801; HR ESI+ finds 524.2794.

17 *α-[(6-Hydroxy)-5-hexynyl] Glycine, Benzyl Ester.* **13**, Benzophenone imine glycine 1-propylpropargyl tetrahydropyranyl ether benzyl ester, (251 mg, 0.15 mmol) was dissolved in 30 mL of diethyl ether and vigorously stirred with 10 mL of aqueous 1.0-N HCl over 2 days. The organic phase was removed and the aqueous phase was washed with diethyl ether 15 mL ×8 until the remaining benzophenone and tetrahydropyranyl alcohol was removed. The aqueous phase was then alkalinized with solid sodium bicarbonate until carbon dioxide bubbling ceased. The solution was further saturated with solid sodium chloride and extracted with diethyl ether 15 mL ×5 that had been passed through an alumina plug. The extracts were dried with magnesium sulfate and evaporated to 112 mg of a clear slightly yellow oil which did not require further purification, 87% yield. [TLC *Rf* = 0.30, 19:1 ethyl acetate/methanol. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.29 (m, 5H), 5.18 (s, 2H), 4.21 (t, J = 2.1 Hz), 3.44 (dd, J_1 = 5.6 Hz, J_2 = 7.3 Hz, 2H), 2.16−2.13 (m, 5H), 1.84−1.75 (m, 1H), 1.68−1.58 (m, 1H), 1.55−1.44 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 135.6, 128.6, 128.3, 85.0, 79.5, 77.3, 77.1, 76.8, 66.6, 53.9, 50.8, 33.6, 24.5, 18.4. m/z $[M+H]^+$ $C_{15}H_{19}NO_3H^+$ calcd MW, 262.1443; ESI+ finds 262.1441. m/z $[M+Na]^+$ $C_{15}H_{19}NO_3Na^+$ calcd MW, 284.1263; HR ESI+ finds 284.1252.

22 *N-o-Nosyl, α-[(7-hydroxy)-6-heptynyl] Glycine, Benzyl Ester.* **18**, Glycine 1-butylpropargyl alcohol ether benzyl ester, (50 mg, 0.18 mmol) was dissolved in 1.5 mL of 2,6-lutidine, and *o*-nosyl-Cl (52 mg, 0.24 mmol) in 0.6 mL of benzene was added dropwise over 30 min with stirring. After being stirred overnight, the reaction contents were partitioned between 20 mL of ethyl acetate and 20 mL of 1.0-N aqueous HCl. The organic phase was then washed with 2× 30 mL of 1.0-N aqueous HCl, then with 20 mL of saturated aqueous NaCl, dried with magnesium sulfate, and evaporated yielding 73 mg of a clear oil requiring no further purification, 87% yield. [TLC *Rf* = 0.65, 9:1 dichloromethane/methanol. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (dd, J_1 = 1.4 Hz, J_2 = 7.7 Hz, 1H), 7.74 (dd, J_1 = 1.2 Hz, J_2 = 7.9 Hz, 1H), 7.58 (dt, J_1 = 1.4 Hz, J_2 = 7.8 Hz, 1H), 7.53 (dt, J_1 = 1.4 Hz, J_2 = 7.8 Hz, 1H), 7.31−7.24 (m, 3H), 7.12−7.06 (m, 2H), 6.08 (d, J = 9.3 Hz, 1H), 4.84 (d, J = 12.2 Hz, 1H), 4.81 (d, J = 12.2 Hz, 1H), 4.16 (m, 3H), 2.12 (m, 2H), 2.03 (s, 1H), 1.85−1.78 (m, 1H), 1.71−1.65 (m, 1H), 1.50−1.38 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 147.5, 134.7, 134.1, 133.6, 132.6, 130.3, 128.7, 128.6, 128.4, 125.7, 85.6, 79.1, 67.4, 56.7, 51.4, 32.6, 27.5, 24.0, 18.4. m/z $[M+NH_4]^+$ $C_{22}H_{24}N_2O_7S_3NH_4^+$ calcd MW, 478.1648; HR ESI+ finds 478.165. m/z $[M+Na]^+$ $C_{22}H_{24}N_2O_7SNa^+$ calcd MW, 483.1202; HR ESI+ finds 483.1210.

26 *N-o-Nosyl, α-([7-Hydroxy-6-heptynyl])-dicobalt hexacarbonyl[μ-[4,5-η;4,5-η]]) Glycine, Benzyl Ester.* **22**, o-Nosyl glycine 1-butylpropargyl alcohol benzyl ester, (65 mg, 0.14 mmol) was dissolved in 4.0 mL of dichloromethane, and dicobalt octacarbonyl (72 mg, 0.21 mmol) weighed into an argon-filled flask was added with stirring. After 2 h the reaction was complete, and the contents were purified by flash chromatography with dichloromethane → 2.5% methanol/dichloromethane gradient yielding 95 mg of a red solid, 91% yield. [TLC *Rf* = 0.85, 9:1 dichloromethane/methanol. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.02 (s, 1H), 7.65 (d, J = 25.8 Hz, 2H), 7.35−7.19 (m, 5H), 6.24 (s, 1H), 5.04−4.85 (m, 4H), 4.26 (s, 1H), 2.85−2.63 (m, 2H), 1.99−0.97 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.7, 170.8, 147.6, 134.7, 134.0, 133.6, 132.8, 130.3, 128.7, 128.6, 128.4, 125.7, 97.4, 96.6, 67.4, 63.4, 56.7, 33.8, 32.7, 30.8, 24.8 m/z $[M+Na]^+$ $C_{28}H_{24}Co_2N_2O_{13}SNa^+$ calcd MW, 768.9561; HR ESI+ finds 768.9558. m/z $[M+NH_4]^+$ $C_{28}H_{24}Co_2N_2O_{13}SNH_4^+$ calcd MW, 764.0007; HR ESI+ finds 764.0013.

30 *1-[o-Nosyl], Cobalt Hexacarbonyl[μ[3,4-η;3,4-η]]-9-carboxybenzyl Azacyclonon-3-yne.* To a 5 mL RB flask was added triphenylphosphine (35 mg, 0.134 mmol) and flushed with argon. Then 1.0 mL of tetrahydrofuran was added, and the contents were chilled to 0 °C. Then, diisopropylazodicarboxylate (29 μL, 0.145 mmol) was added dropwise with stirring over 15 min, and the mixture was allowed to stand chilled at 0 °C for an hour allowing precipitation of the white zwitterion. To the stirring mixture at 0 °C was added **26**, *o*-nosyl glycine 1-butylpropargyl alcohol−dicobalt carbonyl benzyl ester, (10 mg, 0.013 mmol) in 0.5 mL tetrahydrofuran over an hour dropwise. The reaction was let stir for another hour at 0 °C, and rotary evaporated in room temperature water bath to a residue which was extracted with 3:1 hexanes/ethyl acetate and purified by flash chromatography at 3:1 hexanes/ethyl acetate yielding 6 mg, 63% yield. [TLC *Rf* = 0.74, 2:1 hexanes/ethyl acetate; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 (d, J = 8.2 Hz, 1H), 7.54−7.44 (m, 2H), 7.38 (d, J = 8.2 Hz, 1H), 7.28−7.23 (m, 3H), 7.17−7.12 (m, 2H), 5.08 (d, J = 17.5 Hz, 1H), 4.92 (d, J = 12.1 Hz, 1H), 4.83 (d, J = 12.1 Hz, 1H), 4.65 (dd, J_1 = 2.7 Hz, J_2 = 12.2 Hz, 1H) 4.54 (d, J = 17.5 Hz, 1H), 3.15−3.07 (m, 1H), 3.02, 2.93 (m, 1H), 2.13−2.01 (m, 1H), 1.90−1.83 (m, 1H), 1.80−1.67 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.7, 199.4, 170.6, 147.9, 134.9, 133.6, 132.8, 131.5, 130.8, 128.6, 128.5, 128.5, 128.4, 124.0, 95.5, 92.7, 72.6, 67.3, 60.7, 48.7, 32.3, 29.1, 27.0, 25.1, 24.4, 21.7, 14.2. m/z $[2M+NH_4]^+$ $C_{56}H_{44}Co_4N_4O_{24}S_2NH_4^+$ calcd MW, 1473.9459; HR ESI+ finds 1473.9583.

34 *1-[o-Nosyl]-9-carboxybenzyl Azacyclonon-3-yne.* **30**, o-Nosyl cyclononyne dicobalt carbonyl benzyl ester, (50 mg, 0.07 mmol) was dissolved in 8 mL of diethyl ether (which had been eluted through an alumina plug) and chilled to 0 °C. To this stirring solution was added 1.6 g of silica followed by ceric ammonium nitrate (400 mg, 0.70 mmol). After 45 min the entire reaction contents were loaded onto an aminopropylated silica plug, preflushed with diethyl ether, and then eluted with diethyl ether followed by 1:1 hexanes/ethyl acetate. The eluent was evaporated yielding 25 mg of a white solid requiring no further purification, 85% yield. [TLC *Rf* = 0.50, 2:1 hexanes/ethyl

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acetate. ^{1}H NMR (600 MHz, C_6D_6) δ 7.63 (d, J = 7.9 Hz, 1H), 7.08−6.98 (m, 5H), 6.69−6.67 (m, 1H), 6.59−6.55 (m, 1H), 6.48−6.43 (m, 1H), 4.88 (dd, J_1 = 4.6 Hz, J_2 = 11.6 Hz, 1H), 4.86 (d, J = 12.2 Hz, 1H), 4.81 (d, J = 12.2 Hz, 1H), 4.40 (dd, J_1 = 2.5 Hz, J_2 = 18.4 Hz, 1H), 4.28 (m, 1H), 2.14−2.02 (m, 2H), 1.90−1.82 (m, 1H), 1.66 (dd, J_1 = 6.9 Hz, J_2 = 16.9 Hz, 1H), 1.52−1.44 (m, 1H), 1.43−1.34 (m, 1H), 1.28−1.22 (m, 1H), 1.20−1.16 (m, 1H). ^{13}C NMR (125 MHz, C_6D_6) δ 171.0, 148.0, 135.7, 133.9, 132.7, 135.7, 133.9, 132.7, 131.0, 130.6, 128.6, 128.4, 128.0, 127.8, 128.2, 123.8, 94.3, 86.1, 67.8, 60.6, 37.6, 32,6, 25.5, 24.1, 22.9, 22.0, 17.9, 14.2. m/z $[M+Na]^+$ $C_{22}H_{22}N_2O_6SNa^+$ calcd MW, 465.1096; HR ESI+ finds 465.1108.

37 *1-[o-Nosyl]-9-carboxylic Acid Azacyclonon-3-yne.* **34**, *o*-Nosyl cyclononyne benzyl ester, (9 mg, 0.02 mmol) was dissolved in 1.00 mL of tetrahydrofuran and 0.70 mL of water was slowly added. Then 0.2-M aqueous NaOH (0.50 mL, 0.10 mmol) was added slowly dropwise over 10 min. After 12 h the reaction was complete, and the volume of the tetrahydrofuran was evaporated. The mostly aqueous solution was acidified with 1.0-N aqueous HCl by dropwise addition into the stirring solution until pH2−3 was achieved causing precipitation of the carboxylic acid. The aqueous suspension was then extracted with 2 × 10 mL ethyl acetate, dried with magnesium sulfate, and the solvent evaporated leaving an oil. This was purified by flash chromatography with a gradient of 1:1 ethyl acetate/hexanes to ethyl acetate yielding 3 mg of a white powder, 43% yield. [TLC Rf = 0.44, 19:1 ethyl acetate/methanol. ^{1}H NMR (600 MHz, CD_3OD) δ 8.02 (dd, J_1 = 1.3 Hz, J_2 = 7.4 Hz, 1H), 7.70−7.65 (m, 2H), 7.62 (dd, J_1 = 1.7 Hz, J_2 = 7.6 Hz, 1H), 4.49 (d, J = 8.8 Hz, 1H), 4.31 (d, J = 18.5 Hz, 1H), 4.17 (d, J = 18.7 Hz, 1H), 2.23−2.12 (m, 2H), 2.07 (dd, J_1 = 6.9 Hz, J_2 = 18.6 Hz, 1H), 1.74−1.63 (m, 2H), 1.45−1.35 (m, 1H), 1.31−1.18 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 172.3, 148.2 133.6, 131.4, 131.2, 123.6, 93.4, 36.5, 32.2, 31.4, 23.8, 21.1, 21.1,17.2, 12.3. m/z $[M+Na]^+$ $C_{15}H_{16}N_2O_6SNa^+$ calcd MW, 375.0627; HR ESI+ finds 375.0648.

40 *9-Carboxylic Acid Azacyclonon-3-yne.* **37**, *o*-Nosyl cyclononyne carboxylic acid, (8 mg 0.02 mmol) was dissolved in 300 μL of dimethylformamide, then solid K_2CO_3 (25 mg, 0.18 mmol) was added followed by thiophenol (15μL, 0.15 mmol), and the mixture was allowed to stir for 2 days. The solvent was evaporated, the residue partitioned between 3 mL of ethyl acetate and 3 mL of water, and the aqueous phase was washed again with 3 mL ×2 of ethyl acetate. The aqueous phase was then acidified with 1.0-N aqueous HCl dropwise with stirring to pH 2−3. The aqueous phase was evaporated to a residue and then extracted with isopropyl alcohol 3× 3 mL in order to separate the product from the KCl byproduct. The isopropyl alcohol solvent was evaporated, and the residue was purified by flash chromatography with 20:4:1 → 8:4:1 isopropyl alcohol/methanol/saturated aqueous ammonia gradient yielding 2 mg, 62% yield. TLC Rf = 0.33, 8:4:1 isopropyl alcohol/methanol/saturated aqueous ammonia. ^{1}H NMR (600 MHz, D_2O) δ 3.88 (dd, J_1 = 5.6 Hz, J_2 = 6.7 Hz, 1H), 3.80 (dt, J_1 = 2.9 Hz, J_2 = 16.5 Hz, 1H), 3.74 (dd, J_1 = 2.1 Hz, J_2 = 16.6 Hz, 1.0H), 2.25−2.18 (m, 1H), 2.16−2.03 (m, 2H), 1.97−1.90 (m, 1H), 1.86−1.78 (m, 1H), 1.75−1.68 (m, 1H), 1.68−1.52 (m, 2H). ^{13}C NMR (125 MHz, D_2O) δ 175.2, 98.8, 77.5, 60.8, 17.5, 11.9, 25.8, 23.5, 18.6. m/z $[M+H]^+$ $C_9H_{13}NO_2H^+$ calcd MW, 168.1024; HR ESI + finds 168.1046.

Crystallography.

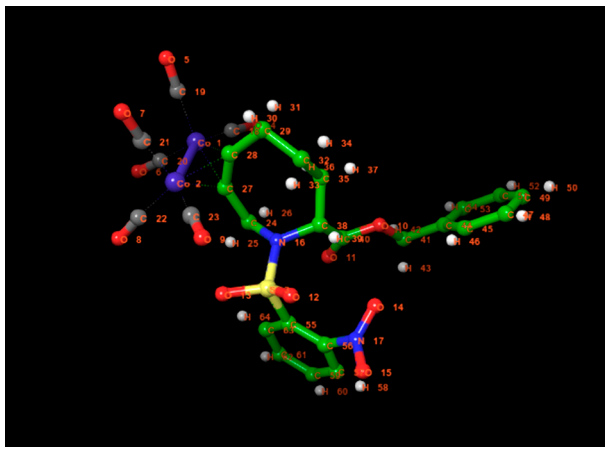

Figure 6. Crystal Structure for **29**, 1-[*o*-nosyl], cobalt hexacarbonyl-[μ[3,4-η;3,4-η]]-9-carboxybenzyl azacyclooct-3-yne; expected connectivity is evident.

CONCLUSIONS

Presented are the syntheses of close structural analogues of proline with an inserted propargyl moiety within a series of ring sizes. Moreover, a synthetic pathway to medium-size ring heterocycloalkynes amenable to wider ranging application mediated by using mild Mitsunobu conditions in tandem with Nicholas reaction for cyclization is introduced. Avoiding the usual harsh acidic conditions for the Nicholas reaction allows improved functional group compatibility. In this application a series of medium-size ring cycloalkyne amino acids were synthesized.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00270.

Experimental details for all compounds (PDF)
Spectra: ^{1}H NMR 1-D and ^{1}H COSY, ^{13}C NMR 1-D, ESI mass spectroscopy (PDF)
Crystal structure for **39** (CIF)

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Author Contributions

The author has been responsible for conceptualization, synthesis lab work, funding acquisition, and writing and editing the drafts.

Notes

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