Synthesis of 3,5-Disubstituted Isoxazoles via 1,3-Dipolar Cycloaddition Reaction between Alkynes and Nitrile Oxides Generated from *O*-Silylated Hydroxamic Acids

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Abstract: In this paper, we report the regioselective synthesis of 3,5-disubstituted isoxazoles by 1,3-dipolar cycloaddition between alkynyl dipolarophiles and nitrile oxide dipoles generated *in-situ* from *O*-silylated hydroxamic acids in the presence of trifluoromethanesulfonic anhydride and NEt_3. Thanks to the mild, metal-free and oxidant-free conditions that this strategy offers, the reaction was successfully applied to a wide variety of alkynyl dipolarophiles, demonstrating the tolerance of this approach to diverse functional groups. In particular, we have shown that the method was compatible with biological molecules such as peptides and peptide nucleic acids (PNA). This protocol constitutes another example of metal-free 1,3-dipolar cycloaddition leading to the regioselective formation of isoxazoles.

Introduction

Identified in countless biologically active derivatives, such as chlorophyll, amino acids, nucleobases and vitamins, five- and six-membered heterocycles are of greatest importance to life, drug discovery and medicinal chemistry.[1] Among them, isoxazoles form a major class of five-membered heterocycles with two heteroatoms (Figure 1).[2] Isoxazoles are key pharmacophores occurring in many natural products, *e.g.* ibotenic acid **1** and muscimol **2**;[3] in a variety of bioactive compounds, such as anti-inflammatories **3** and **4**,[4] monoamine oxidase inhibitor **5**,[5] penicillin antibiotic **6**,[6] and herbicidal isoxaflutole **7**.[7] Isoxazoles have other biological properties as antitubulin, antinociceptive and anticancer.[8] They also find applications in material science, *e.g.* molecular switches and polymer syntheses.[9] Furthermore, these heterocycles constitute an important organic synthetic tool. Indeed, although isoxazoles are hydrolytically stable, they can be cleaved under reducing or basic conditions, leading to different important latent functionalities, namely 1,3-dicarbonyl compounds **8**, enaminones **9**, and β-amino carbonyls **10** (Figure 1).[2, 10] As a result, many research groups have used isoxazole derivatives as masked functions in synthetic strategies towards heterocyclic compounds and natural products.[11] Isoxazoles, and their partially saturated analogues, can be prepared using diverse synthetic strategies.[12] Among them, the 1,3-dipolar cycloaddition reaction between nitrile oxides and corresponding alkenyl and alkynyl dipolarophiles is probably the most convenient, attractive and direct approach, via a preferred *in-situ* preparation of the dipoles.[1b, 2, 12a, 13] Indeed, as *Quilico* demonstrated in 1970, nitrile oxides in the presence of none or poor trapping agents, readily dimerize to furoxans.[14] They can also dimerize to 1,2,4-oxadiazole 4-oxide in the presence of NEt_3.[13a, 13b, 15]

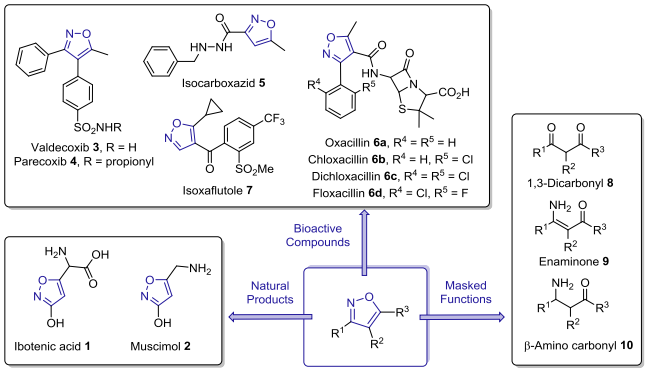

Figure 1. Examples of natural products (**1-2**) and bioactive compounds (**3-7**) bearing an isoxazole function. Isoxazoles can also be used as masked functional groups for various synthetic strategies *e.g.* towards **8-10**.

Several methods towards the *in-situ* generation of nitrile oxides have thus been developed, two of which are by far the most popular and well-established protocols. The first approach, namely the *Mukaiyama* method (Figure 2, *Method a*), involves the dehydration of nitroalkanes by aryl isocyanates in the presence of NEt_3.[13c] The use of $POCl_3$, $MeSO_2Cl$ and BzCl as dehydrating agents are improved variants of the original *Mukaiyama* procedure.[13a, 13b, 13d, 16] The action of DMAP and Boc_2O[17] or DMTMM[18] on nitroalkanes was also successfully used to give nitrile oxide dipoles. The second method relies on the base-mediated dehydrochlorination of hydroximoyl chlorides to yield the desired nitrile oxides (Figure 2, *Method b*).[13l] This procedure is often carried out as a halogenation/dehydrohalogenation reaction on the parent aldoximes, especially for unstable hydroximoyl chlorides.[13a, 13b] NCS, *tert*-BuOCl or NaOCl in the presence of NEt_3, or chloramine-T without additional bases, are frequently employed in these procedures.[13e, 13f] On top of these approaches, the direct oxidation of aldoximes through the use of MnO_2[19] or organic hypervalent iodine reagents[20] is also a promising route to the *in-situ* generation of nitrile oxides (Figure 2, *Method c*). Another strategy was described by *Carreira* and co-workers in 2000.[21] It involves the treatment of *O*-silylated hydroxamic acids with Tf_2O in the presence of NEt_3 (Figure 2, *Method d*). In 2019, Dai *et al*. reported a novel direct Csp^3-H bond functionalization of 2-methyl ketones and 2-methylquinoline with

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tert-BuONO, generating nitrile oxide *in-situ* via a radical mechanism (Figure 2, *Method e*).[22]

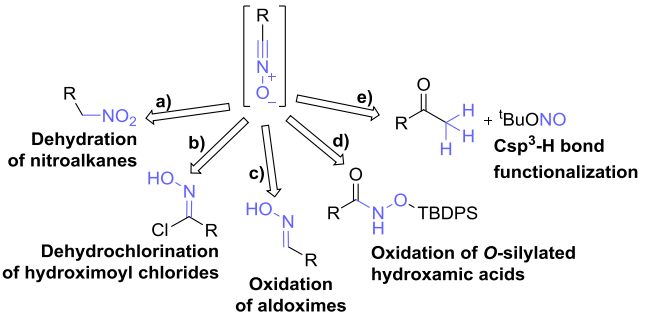
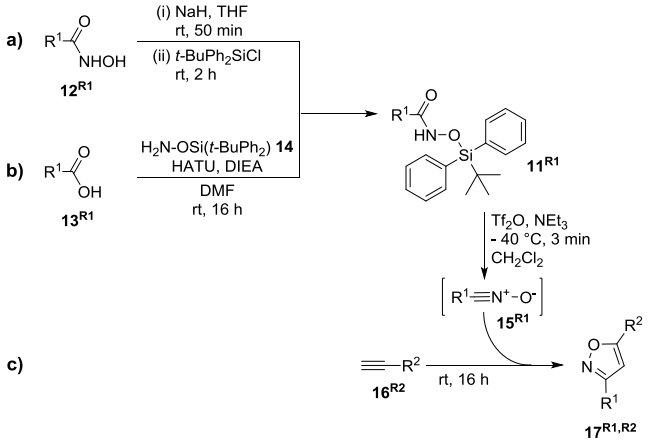


Figure 2. Overview of the synthetic approaches for preparing nitrile oxides *in-situ*: a) dehydration of nitroalkanes; b) dehydrochlorination of hydroximoyl chlorides; c) oxidation of aldoximes; d) oxidation of *O*-silylated hydroxamic acids; and e) Csp^3-H bond functionalization of 2-methylketones with *tert*-BuONO.

Although the cycloaddition between nitrile oxides and alkenyl derivatives is well-developed, the reaction with alkynyl dipolarophiles is usually affected by side reactions, often leading to byproducts and low yields. This is due to the relatively inert character of the triple bond. Following the Cu(I)-catalyzed azide-alkyne cycloaddition protocol,[23] it was also demonstrated that the addition of Cu(I)-[24] or Ru(II)-based[25] catalysts to a mixture of alkynes and nitrile oxides, the latter species being generated *in-situ* respectively from the parent aldoximes and hydroximoyl chloride, led to the formation of 3,5-di or 3,4-di and 3,4,5-trisubstituted isoxazoles in high yields. Among the metal-free protocols, the group of *Heaney* conjugated aryl moieties with DNA on solid phase through a 1,3-dipolar cycloaddition between nitrile oxide and alkynyl moieties.[26] In their approach, nitrile oxide was generated *in-situ* by dehydrogenation of aryl aldoxime. In 2011, the group of *Van Delft* described an efficient phenyliodine bis(trifluoroacetate) (PIFA)-mediated synthesis of isoxazoles.[20b] In a parallel avenue, *Kankala et al*. reported in 2011 the nucleophilic organocarbene-catalyzed 1,3-dipolar cycloaddition of nitrile oxides with alkynes.[27] In 2014, the group of *Pal* reported the use of polyethylene glycol to facilitate the 1,3-dipolar cycloaddition of benzoylnitromethane and ethyl 2-nitroacetate with terminal alkynes leading to isoxazoles under green conditions.[28] Recently, the Csp^3-H metal-free radical functionalization/cycloaddition cascade from ketones, alkynes and *tert*-BuONO proposed by Dai *et al.* allowed the synthesis of 3-acyl and 3-quinoline isoxazoles in moderate to good yield.[22]

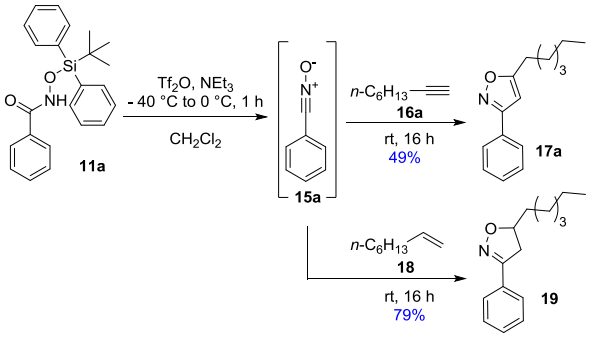
While studying metal-free methodologies for the bioconjugation of structures of biological interest with functional organic dyes,[29] our group became interested in developing an alternative bioconjugation approach that would use the 1,3-dipolar cycloaddition reaction between a nitrile oxide and an alkynyl derivative to produce isoxazole linkers. During these endeavors, we focused our attention on the *Carreira's* protocol,[21] that produces isoxazolines *via* cycloaddition between an alkenyl derivative and a nitrile oxide, the latter being produced *in-situ* from *O*-silylated hydroxamic acids upon treatment with Tf_2O and NEt_3 (Figure 2, *Method d*). In addition to the mild, metal-free and oxidant-free conditions that this strategy offers, we conjectured that the straightforward preparation of the parent *O*-silylated hydroxamic acids **11^{R1}** could also be easily adapted on a large variety of substrates. Indeed, *O*-silylated hydroxamic acids can be readily obtained from the corresponding hydroxamic acids **12^{R1}** by silylation with *tert*-butyl(chloro)diphenylsilane in the presence of NaH (Scheme 1a). They can also be prepared from the corresponding carboxylic acids **13^{R1}** by reaction with *O*-silylated hydroxylamine **14**[30] following activation of the carboxylic acid moiety with HATU in the presence of DIEA (Scheme 1b). To our surprise, this method has not been used to prepare isoxazoles. It is with this aim that herein we report the successful adaptation of the *Carreira*'s protocol to the regioselective preparation of 3,5-disubstituted isoxazoles (Scheme 1c) through the 1,3-dipolar cycloaddition reaction between *in-situ* generated nitrile oxides (**15^{R1}**) and alkynyl dipolarophiles **16^{R2}**.



Scheme 1. Synthetic protocol proposed in this work: formation of *O*-silylated hydroxamic acids **11^{R1}** a) from hydroxamic acid derivatives **12^{R1}**, and b) from parent acids **13^{R1}**. Yields were 72% and 44% for R^1 = Ph, respectively. c) Optimized 1,3-dipolar cycloaddition developed in this work for preparing 3,5-disubstituted isoxazoles **$17^{R1,R2}$**.

Results and Discussion

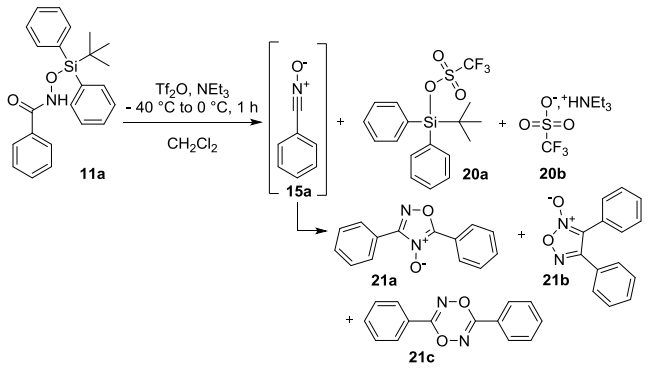
We commenced our studies with the investigation of the basic reactivity of alkynes respect to alkenes in the 1,3-dipolar cycloaddition reaction with *O*-silylated hydroxamic acid **11a** (R^1 = Ph). This was achieved using aliphatic compounds 1-octyne **16a** and 1-octene **18**, following *Carreira*'s procedure (Scheme 2).[21] Hence, after dropwise addition of 1 M Tf_2O in CH_2Cl_2 to a solution of **11a** and NEt_3 in CH_2Cl_2 at -40 °C, the reaction mixture was allowed to stir for 1 h at 0 °C, after which an 8-fold excess of dipolarophile[31] **16a** or **18** was added, and the solution stirred overnight at rt. Desired isoxazole **17a** and isoxazoline **19** were successfully synthesized in 49% and 79% isolated yield, respectively. The 30% difference in yield between the two reactions confirmed the expected lower reactivity of alkynes.[32]



Scheme 2. Evaluation of the reactivity of 1-octyne **16a** respect to 1-octene **18** in the 1,3-dipolar cycloaddition reaction with nitrile oxide **15a**, generated *in-situ* from *O*-silylated hydroxamic acid **11a**.

Next, we attempted to improve the cycloaddition yield with 1-octyne **16a**.[33] First, all side-products were isolated and compared to those obtained in a second trial in which no dipolarophile had been added to the reaction mixture. We observed that these side-products (Scheme 3, **20/21**), of which structures **21** were proposed with the support of literature,[13a, 15] were identical in the absence and in the presence of 1-octyne. This observation suggested that the side-reactions mainly derive from nitrile oxide **15a** and are independent on the dipolarophile. With this information in hand, we attempted the optimization of the reaction, with the idea of favoring the intermolecular cycloaddition reaction over the other side-reactions. Several conditions were explored, mainly focusing on the nitrile oxide generation.[33] Parameters such as the excess of the dipolarophile, time, temperature, the order of addition of the reagents, as well as the concentration of the reaction mixture were studied. Although none of these tests gave improved yields, we could conclude that: *(i)* the side-products mainly derived from the reaction of nitrile oxide **15a** with itself or with **20a** (Scheme 3); *(ii)* these side-reactions do not take place below 0 °C; and *(iii)* they are faster than the 1,3-dipolar cycloaddition with 1-octyne at rt. In light of these observations, we decided to monitor the generation of nitrile oxide at different temperatures, and noticed that it is formed within three minutes at -40 °C. This observation allowed us to improve the protocol. Namely, the reaction mixture was allowed to stir only for 3 min at -40 °C after the addition of Tf_2O to a solution of **11a**, before it was cannulated over 1 h onto neat 1-octyne **16a** at rt. The solution was then stirred overnight at rt (Scheme 1c). Following this procedure, desired isoxazole **17a** was formed in 75% yield, with an overall increase of 26%. To the best of our knowledge, this is one of the most efficient synthetic strategies to form 5-hexyl-3-phenylisoxazole **17a**.[34] Similar yields were obtained either using the base-mediated conversion of propargylic *N*-hydroxylamines, through a detosylative 5-*endo*-*dig*-cyclisation,[34a] or exploiting hypervalent iodine to generate *in-situ* the nitrile oxide species.[34b] Having in our hands the optimal conditions for our transformation, we extended the protocol to a variety of *O*-silylated hydroxamic acids $\mathbf{11^{R1}}$ and of alkynyl substrates $\mathbf{16^{R2}}$ (Scheme 4).

We started by studying the effect of electron withdrawing groups (EWG) and electron donating groups (EDG) on the alkynyl reagent.[32] Reactions were carried out between *O*-silylated hydroxamic acid **11a** and aromatic alkynes: ethynylbenzene **16b** as a reference, electron-poor 1-ethynyl-4-(trifluoromethyl) benzene **16c** and electron-rich 4-ethynyl-*N*,*N*-dimethylaniline **16d**. When adding the alkyne in one batch onto benzonitrile oxide (according to Scheme 2),[21] both activated dipolarophiles **16c** and **16d** gave the corresponding desired isoxazoles in higher yield than reference ethynylbenzene **16b** (~65% isolated yield respect to 54%).



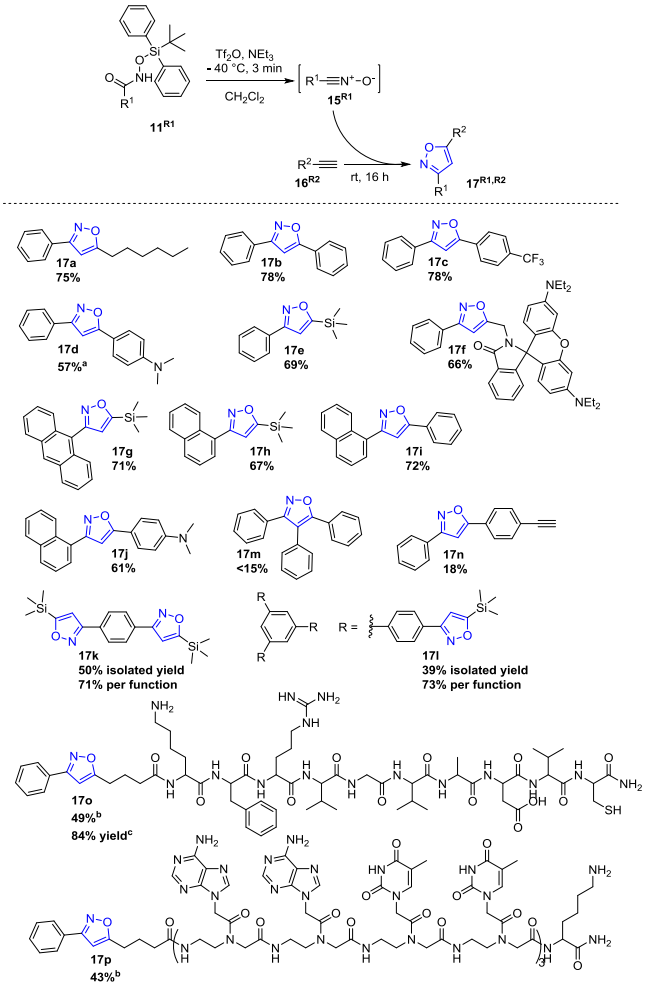
Scheme 3. Reaction side-products **20** are formed during the formation of nitrile oxide. By-products **21** derive from the dimerization of nitrile oxides. Reported structures **21** are proposals which were assigned with the support of literature.[13a, 15]

The cycloaddition reaction yields were increased with ethynylbenzene **16b** and 1-ethynyl-4-(trifluoromethyl) benzene **16c** by cannulating the nitrile oxide into a solution of the dipolarophile (Scheme 1c). Indeed, both dipolarophiles afforded desired isoxazoles **17b** and **17c** in 78% of yield. On the other hand, 4-ethynyl-*N*,*N*-dimethylaniline **16d** seemed to have decomposed to some extent under these conditions, thus giving an yield of 57%. Although the isolated yield for 3,5-diphenyl isoxazole **17b** are slightly inferior to the protocols using PIFA (90%)[20b] or a mixture of KCl and oxone in H_2O (87%),[35] our method gave similar results as those obtained with Cu(I)-catalyzed synthesis of isoxazoles (72%),[24] as well as to other metal-based oxidative[19, 20c, 20d, 34b] and dehydrating strategies.[17]

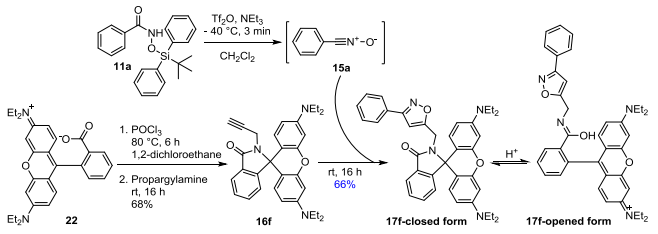
Using the optimized procedure, we then evaluated the compatibility of the reaction conditions with other functional groups. The protocol afforded 3-phenyl-5-(trimethylsilyl)isoxazole **17e** in 69% when starting from *O*-silylated hydroxamic acid precursor **11a** and ethynyltrimethylsilane **16e**. The reaction was also successfully applied to ethynylrhodamine **16f** (Scheme 5).[36] The 1,3-dipolar cycloaddition reaction with nitrile oxide **15a** led to the formation of desired isoxazole **17f** in 66%. Upon formation of desired cycloadduct **17f**, the ring-opening of the spirolactam moiety was observed, as described in Scheme 5.[37] Considering its application as molecular sensor, the result obtained with rhodamine B **22** was particularly interesting as this strategy offers a new route of functionalization of the fluorophore.[38] Indeed, modification of the *N*-terminus of the spiroamide moiety of **16f** with a conveniently functionalized dipole precursor, could allow its conjugation to various receptors for the targets of interests.

The 1,3-dipolar cycloaddition reaction was tested using ethynyltrimethylsilane **16e** with polycyclic aromatic dipoles **11b** and **11c**, namely with an anthracenyl and a naphthyl core, respectively. The corresponding *O*-silylated hydroxamic acids were synthesized according to Scheme 1b. The desired isoxazoles **17g** and **17h** were respectively generated in 71% and 67% isolated yields. The 1,3-dipolar cycloaddition reaction was also performed between naphthyl precursor **11c** and ethynylbenzene **16b** as well as 4-ethynyl-*N*,*N*-dimethylaniline **16d**.

Corresponding isoxazoles **17i** and **17j** were formed in 72% and 61% yield, respectively.

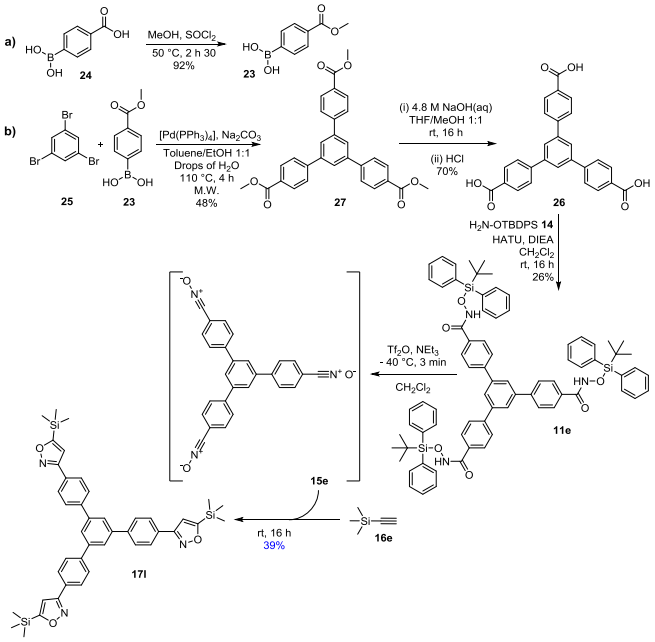


Scheme 4. Scope of this work: the optimized protocol for the 1,3-dipolar cycloaddition reaction between alkynyl dipolarophiles **16^{R2}** and nitrile oxide **15^{R1}** generated *in-situ* from *O*-silylated hydroxamic acid **11^{R1}** was applied to a variety of substrates leading to diverse isoxazoles **17^{R1,R2}**. Isolated yields are reported. [a] 65% with original *Carreira*'s protocol. [b] Measured %area of desired cycloadduct in the RP-HPLC chromatogram of the crude reaction mixture. [c] Calculated from 49% conversion, as determined from the RP-HPLC chromatogram of the crude reaction mixture.



Scheme 5. Synthesis of ethynylrhodamine **16f** and further 1,3-dipolar cycloaddition reaction with nitrile oxide **15a**. Upon formation of desired isoxazole **17f**, the ring-opening of the spirolactam form was observed in the presence of acid.

The reaction was also extended to di- and tri-topic substrates, starting with the simple di-substitution pattern consisting of a 1,4-benzene core. The corresponding *O*-silylated hydroxamic acid **11d** was synthesized according to Scheme 1b from terephthalic acid in 48% isolated yield, corresponding to 69% per functionality. The 1,3-dipolar cycloaddition reaction with ethynyltrimethylsilane **16e** provided desired **17k** in 50% yield, corresponding to 71% yield per hydroxamic acid group. Next, we focused on a tris-1,3,5-benzene substituted core pattern. *O*-silylated hydroxamic acid derivative **11e** was synthesized in four steps (Scheme 6). First, 4-methoxycarbonylphenylboronic acid **23** was prepared by esterification of 4-carboxyphenylboronic acid **24** with MeOH in $SOCl_2$.[39] Reaction with 1,3,5-tribromobenzene **25** in the presence of a catalytic amount of $[Pd(PPh_3)_4]$ and Na_2CO_3 under microwave conditions,[40] gave desired tricarboxylic acid derivative **26** after saponification with aqueous NaOH of resulting tris-ester **27**.[41] Activation of tris-carboxylic acid **26** with HATU in the presence of DIEA, followed by reaction with *O*-silylated hydroxylamine **14**, afforded desired nitrile oxide precursor **11e**. Finally, subsequent cycloaddition reaction with ethynyltrimethylsilane **16e** provided desired tris-isoxazole **17l** in 39% yield, *i.e.* 73% yield per functionality.

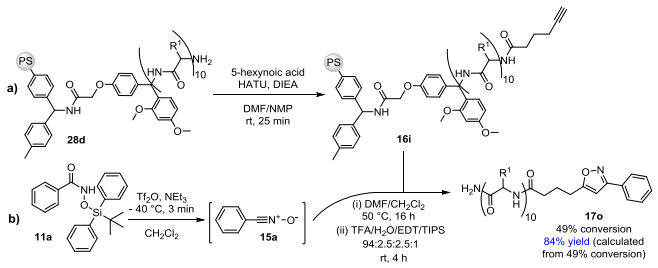


Scheme 6. Synthesis of tris-*O*-silylated hydroxamic acid precursor **11e** and subsequent 1,3-dipolar cycloaddition reaction with ethynyltrimethylsilane **16e**.

Lastly, we have studied the cycloaddition reaction between *O*-silylated hydroxamic acid **11a** and 1,2-diphenylethyne **16g** and with 1,4-diethynylbenzene **16h**. 3,4,5-triphenylisoxazole **17m** was obtained in less than 15% yield. This is in line with the limited reactivity of internal alkynes with nitrile oxides, as it is well established that isoxazoles would exclusively be obtained from electron-deficient internal acetylenic dipolarophiles.[2b, 42] On the other hand, only one of the two alkynyl moiety of 1,4-diethynylbenzene **16h** was successfully converted to isoxazole, leading to derivative **17n** in 18% yield. We explained this result by considering the constraint imposed by 1,4-diethynylbenzene **16h** to work with an excess of nitrile oxide, favoring decomposition of the latter into by-products over its reaction with alkynyl dipolarophile **16h** (Scheme 3).

The 1,3-dipolar cycloaddition reaction was finally used to functionalize peptide and peptide nucleic acid (PNA) species. Both were synthesized on a solid phase semi-automatic peptide synthesizer (*Focus XC*), using a classical 9-fluorenylmethyl carbamate (Fmoc) solid phase procedure (Scheme SI1) on a Rink

Amide MBHA resin, with the required amino acids (aa) or PNA monomers.[43] A peptide sequence (KFRVGVADVC) displaying potential function in antitumor therapies[44] was selected, and synthesized (Section 6 in the SI). Functionalization at the *N*-terminal position with an acetylenic moiety was subsequently achieved to allow the 1,3-dipolar cycloaddition with nitrile oxides generated from *O*-silylated hydroxamic acid **11a** (Scheme 7). The ethynyl moiety was introduced in the sequence by coupling 5-hexynoic acid with the relevant peptide precursor **28d** under the standard solid phase synthesis conditions (Scheme 7a). The 1,3-dipolar cycloaddition reaction with nitrile oxide **15a** was then performed on solid phase (Scheme 7b). To this end, a 10-fold excess of nitrile oxide, generated *in-situ* from *O*-silylated hydroxamic acid **11a** with Tf_2O in the presence of NEt_3 at -40 °C, was cannulated at the same temperature onto a suspension of resin-bound ethynyl-functionalized peptide **16i** in DMF, heated at 50 °C for 16 h. This was followed by cleavage from the resin, complete deprotection of the side chains using an acidic cleavage mixture TFA/H_2O/EDT/TIPS 94:2.5:2.5:1, precipitation with Et_2O, and analysis of the resulting white solids by reverse phase HPLC (RP-HPLC). The RP-HPLC analysis of crude **17o** displayed two major products, namely isoxazole **17o**, and cleaved starting material ethynyl-peptide **16i**. Incomplete conversion was observed likely due to the favored side-reactions of nitrile oxide over the reaction with the alkynyl moieties, when the former is in excess. Yet, the 1,3-dipolar cycloaddition reaction was shown to be compatible with peptidic structures and solid phase synthesis. Based on the analytical RP-HPLC chromatogram of the crude reaction mixture, 49% conversion of *N*-terminal ethynyl-peptide **16i** was obtained, of which 41% corresponded to desired cycloadded peptide **17o**, and 8% to unidentified side-products, thereby resulting in 84% yield of peptide-isoxazole conjugate.



Scheme 7. a) Synthesis of resin-bound ethynyl-functionalized peptide **16i**; b) 1,3-dipolar cycloaddition reaction on solid phase between **16i** and nitrile oxide **15a**. The yield was calculated based on RP-HPLC chromatogram (%area) of the crude reaction mixture and on the measured 49% conversion. PS = polystyrene; R^1 = amino acid side chain. Sequence: KFRVGVADVC.

Next, the reaction was attempted on self-complementary PNA (sequence: $(AATT)_3$-Lys), which was synthesized from the relevant Fmoc/benzyloxycarbonyl (Cbz)-protected PNA monomers. Solid phase synthesis, terminal functionalization with an acetylenic moiety and reaction with nitrile oxide **15a** following the same strategies as that described above for ethynyl-peptide **16i**, gave PNA-isoxazole conjugate **17p** formed in 43% (yield calculated on the %area from the RP-HPLC chromatogram of the crude reaction mixture). No trace of ethynyl-functionalized PNA **16j** were detected.[33]

Conclusions

In conclusion, we have successfully demonstrated the catalyst- and oxidant-free regioselective synthesis of 3,5-disubstituted isoxazoles by 1,3-dipolar cycloaddition reactions between alkynyl dipolarophiles and nitrile oxides, with the latter reagent being produced *in-situ* under mild conditions. The method is experimentally straightforward and convenient as it makes use of stable crystalline *O*-silylated hydroxamic acids dipole precursors readily synthesized from the corresponding hydroxamic or carboxylic acids. Through the application of the protocol to a variety of dipoles and dipolarophiles, we have observed that the mildness of this approach provides a tolerance to diverse functional groups as different 3,5-disubstituted isoxazoles were successfully synthesized in moderate to good yields. In particular, we have shown that the method was compatible with biological molecules such as peptides and PNA,[45] thus opening the way to the biorthogonal applications.[46] An isoxazole derivative of rhodamine B was also successfully formed, indicating that the strategy could provide a promising new functionalization route towards labelling and sensing applications.

Experimental Section

General Information: Chemicals were purchased from *Sigma Aldrich*, *Acros Organics*, *Fluorochem, TCI*, *aapptec*, *carbosynth*, and *ABCR,* and were used as received from the commercial suppliers. Resins for solid phase synthesis were purchased from *Peptides International* and *aapptec*. Solvents were purchased from *Sigma Aldrich* and *Acros Organics*. Deuterated solvents were purchased from *Eurisotop*. General solvents were distilled *in vacuo*. Anhydrous CH_2Cl_2 was distilled from phosphorus pentoxide. Anhydrous DMF was purchased from *Acros Organics*. Low temperature baths were prepared using different solvent mixtures depending on the desired temperature: -40 °C with CH_3CN/liquid N_2, -10 °C with ice/brine, and 0 °C with ice/H_2O. Anhydrous conditions were achieved by drying *Schlenk* lines, 2-neck flasks or 3-neck flasks by flaming with a heat gun under vacuum and then purging with argon. The inert atmosphere was maintained using argon-filled balloons equipped with a syringe and needle that was used to penetrate the silicon stoppers used to close the flasks' necks. The addition of liquid reagents was done by means of dried plastic syringes or by cannulation, using standard inert atmosphere techniques. Microwave reactions were performed on a *Biotage AB Initiator* microwave instrument producing controlled irradiation at 2.45 GHz. Solid phase peptide syntheses were performed on a semi-automatic *FOCUS XC* peptide synthesizer, coming with a computer/control system from *aapptec*. Reactions were monitored by thin layer chromatography (TLC) using pre-coated aluminum sheets with 0.20 mm *Machevery-Nagel* Alugram SIL G/UV_{234} with fluorescent indicator UV_{254} or UV_{366}. Components were visualized by illumination with short-wavelength UV light. All products were purified by flash column chromatography on *Grace* silica gel 60 (particle size 40-63 µm). Peptide and PNA oligomers were analyzed and purified by high performance liquid chromatography (HPLC) on a *Varian 940-LC* liquid chromatograph system with a *Varian Pursuit C18, 5 µm, 250 × 4.6 mm* analytical column and a *Varian Pursuit C18, 5 µm, 250 × 21.2 mm* preparative column. 0.1% TFA in H_2O and 0.1% TFA in CH_3CN were used as eluents in all cases. Lyophilisation was performed on a *Christ Freeze Dryer ALPHA 2-4 LD_{plus}*, connected to a *Vacuubrand Chemistry-HYBRID-pump*, with an ice condenser temperature of approx. -85 °C and a vacuum of approx. 2.10^{-3} mbar. ^1H and ^{13}C NMR spectra were obtained on a 400 MHz NMR *(Jeol JNM EX-400)*. Chemical shifts were reported in ppm according to tetramethylsilane using the solvent residual signal as an internal reference

($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.16 ppm; CD_2Cl_2: δ_H = 5.32 ppm, δ_C = 53.84 ppm; DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.52 ppm). Coupling constants (*J*) were given in Hz and were averaged. Resonance multiplicity was described as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *quin* (quintet), *m* (multiplet), *br* (broad signal), *dd* (doublet of doublets). Carbon spectra were acquired with a complete decoupling for the proton.

General Experimental Procedure for the Preparation of *O*-(tert-Butyldiphenylsilyl)hydroxamic acids (11) – *Hydroxamic acid route (Method A)*: A solution of benzhydroxamic acid (1 eq) in THF was treated at 0 °C with NaH (60% in mineral oil, 2 eq) in two portions at 20 min intervals, under an inert atmosphere of argon. After 30 min, when all H_2 gas evolution had stopped, the solution was cooled to 0 °C, and *tert*-butylchlorodiphenyl silane (1.1 eq) was added dropwise over 30 min. The reaction mixture was then allowed to warm up to rt and was stirred for additional 2 h. It was subsequently diluted with H_2O and extracted with EtOAc. The organic extracts were washed with H_2O and dried over anhydrous Na_2SO_4, filtered and concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography to afford desired product **11**. With this procedure, we prepared derivative **11a**.

General Experimental Procedure for the Preparation of *O*-(tert-Butyldiphenylsilyl)hydroxamic acids (11) – *Carboxylic acid route (Method B):* To a suspension of benzoic acid (1 eq) in anhydrous DMF were added DIEA (2 eq) and HATU (1.1 eq), in this order, under an inert atmosphere of argon. The resulting reaction mixture was stirred for 30 min at rt, after which *O*-silylated hydroxylamine **14** (1.5 eq) was added at 0 °C and the resulting solution stirred overnight (approx. 16 h) at rt. It was then concentrated to dryness *in vacuo* to yield a solid residue, which was dissolved in CH_2Cl_2 and washed with H_2O. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography to afford desired product **11**. With this procedure, we prepared derivatives **11a-e**.

***N*-((tert-Butyldiphenylsilyl)oxy)benzamide (11a):**

Synthesis according to Method A: Yield 72% (492 mg) of desired product **11a** as a white solid. *Synthesis according to Method B:* Yield 44% (2.69 g, white solid). R_f = 0.42 (Cyclohexane(Cy)/EtOAc 95:5). m.p. 134-137 °C; ^{1}H NMR (400 MHz, DMSO-d_6, *denotes rotamer peaks): δ 11.40 & 10.92* (s, 1H, N*H*), 7.76 (m, 4H, C*H*), 7.53-7.37 (m, 11H, C*H*), 1.11 (s, 9H, C*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.5, 135.5, 132.7, 132.1, 131.4, 130.1, 128.4, 127.6, 127.2, 26.8, 19.2. All other spectroscopic and analytical properties were identical to those reported in the literature.[21]

***N*-((tert-Butyldiphenylsilyl)oxy)anthracenyl-9-carboxamide (11b), synthesis according to Method B:** Yield 41% (351 mg) of desired product **11b** as a yellow solid. R_f = 0.62 (CH_2Cl_2/MeOH 95:5). m.p. 91-94 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (s, 1H, N*H*), 8.60 (s, 1H, C*H*), 8.04 (m, 2H, C*H*), 7.88 (m, 4H, C*H*), 7.57 (m, 2H, C*H*), 7.51-7.44 (m, 6H, C*H*), 7.30-7.23 (m, 4H, C*H*) 1.17 (s, 9H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.7, 135.9, 131.4, 130.4, 130.3, 129.7, 128.2, 127.8, 127.7, 126.4, 125.5, 125.1, 26.9, 19.2 (one of the quaternary aromatic carbons overlaps with another signal); IR (cm^{-1}): ν 507.3, 698.7, 709.2, 727.8, 737.9, 807.9, 888.5, 1060.0, 1116.1, 1427.5, 1471.0, 1498.4, 1648.9, 2856.9, 2929.8, 2955.5, 3052.6, 3200.4; MS (APCI-HR-MS): Found 476.2043 $[M+H]^+$, $C_{31}H_{29}NO_2Si$ requires = 476.2046.

***N*-((tert-Butyldiphenylsilyl)oxy)-1-naphthamide (11c), synthesis according to Method B:** Yield 48% (237 mg) of desired product **11c** as a white solid. R_f = 0.45 (Cy/EtOAc 93:7). m.p. 164-167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H, N*H*), 7.95 (m, 1H, C*H*), 7.89 (m, 1H, C*H*), 7.81 (m, 4H, C*H*), 7.51-7.42 (m, 9H, C*H*), 7.36-7.34 (m, 1H, C*H*), 7.27 (d, *J* = 6.9 Hz, 1H, C*H*), 1.14 (s, 9H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.5, 135.8, 132.9, 131.9, 131.7, 130.2, 130.1, 129.9, 128.0, 127.6, 126.6, 126.2, 125.5, 125.1, 124.7, 26.9, 19.2; IR (cm^{-1}): ν 503.2, 526.6, 567.3, 593.9, 615.1, 623.3, 689.0, 701.7, 711.6, 734.2, 754.1, 782.6, 805.3, 822.8, 899.5, 1054.6, 1116.5, 1427.2, 1512.4, 1651.9; MS (ESI-HRMS): Found 426.1882 $[M+H]^+$, $C_{27}H_{28}NO_2Si$ requires = 426.1884; Found 448.1703 $[M+Na]^+$, $C_{27}H_{27}NNaO_2Si$ requires = 448.1703.

***N*^{1},*N*^{4}-bis((tert-Butyldiphenylsilyl)oxy)terephthalamide (11d), synthesis according to Method B:** Yield 48% (581 mg; 69% per functionality) of desired product **11d** as a white solid. R_f = 0.51 (Cy/EtOAc 90:10). m.p. decomposition at 121-123 °C; ^{1}H NMR (400 MHz, DMSO-d_6, *denotes rotamer peaks): δ 11.47 & 11.13* (s, 2H, N*H*), 7.73-7.72 (m, 8H, C*H*), 7.49-7.38 (m, 16H, C*H*), 1.09 (s, 18H, C*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 135.4, 134.6, 132.3, 130.0, 127.6, 126.8, 26.9, 19.2, quaternary carbonyl carbon signal was not observed; IR (cm^{-1}): ν 507.2, 615.9, 622.7, 697.1, 710.9, 757.7, 764.8, 822.6, 852.8, 894.8, 1013.1, 1031.2, 1116.0, 1167.2, 1194.4, 1261.1, 1275.7, 1312.3, 1362.1, 1427.7, 1471.1, 1486.0, 1516.3, 1651.8, 2859.4, 2955.5, 3190.5; MS (ESI-HRMS): Found 673.2905 $[M+H]^+$, $C_{40}H_{45}N_2O_4Si_2$ requires = 673.2912.

***N*^{4},*N*$^{4''}$-bis((tert-Butyldiphenylsilyl)oxy)-5'-(4-(((tert-butyldiphenylsilyl)oxy)carbamoyl)phenyl)-[1,1':3',1''-terphenyl]-4,4''-dicarboxamide (11e,) synthesis according to Method B:** Yield 26% (104 mg; 64% per functionality) of desired product **11e** as a white solid. R_f = 0.39 (CH_2Cl_2). m.p. 104-107 °C; ^{1}H NMR (400 MHz, DMSO-d_6, *denotes rotamer peaks): δ 11.48 & 11.04* (s, 3H, N*H*), 7.94 (s, 3H, C*H*), 7.91 (d, *J* = 8.2 Hz, 6H, C*H*), 7.81-7.74* (m, 13.5H, C*H*), 7.64* (d, *J* = 8.2 Hz, 4.5H, C*H*), 7.49-7.40 (m, 18H, C*H*), 1.13 (s, 27H, C*H*); ^{13}C NMR (100 MHz, DMSO-d_6, *denotes rotamer peaks): δ 166.0, 142.6, 140.7, 135.6, 135.2, 134.8*, 134.5*, 133.5*, 132.1, 131.8*, 130.1, 129.7*, 129.2*, 127.7, 127.6, 127.5*, 127.3*, 127.1, 126.2*, 125.1, 27.2*, 26.8, 26.5*, 19.2; IR (cm^{-1}): ν 486.8, 502.4, 612.4, 621.6, 649.4, 697.9, 738.7, 760.0, 797.9, 821.9, 876.7, 909.9, 952.9, 998.3, 1013.8, 1035.5, 1106.78, 1113.9, 1158.7, 1187.5, 1233.5, 1291.5, 1314.0, 1362.5, 1392.1, 1409.1, 1427.4, 1441.4, 1371.6, 1487.7, 1526.8, 1588.4, 1609.8, 1681.5, 1899.7, 2857.0, 2893.2, 2930.3, 3048.8, 3071.8, 3192.4, 3408.3; MS (ESI-HRMS): Found 1198.5030 $[M+H]^+$, $C_{75}H_{76}N_3O_6Si_3$ requires = 1198.5036; Found 1199.0054 $[2M+2H]^{2+}$, $C_{150}H_{152}N_6O_{12}Si_6$ requires = 1199.0053.

***O*-(tert-Butyldiphenylsilyl)hydroxylamine (14):** To a stirred suspension of hydroxylamine hydrochloride (500 mg, 7.19 mmol) in anhydrous CH_2Cl_2 (20 mL) was added NEt_3 (1.60 g, 2.21 mL, 15.83 mmol) under an inert atmosphere of argon. The mixture was allowed to stir for 1 h at rt. Neat *tert*-butylchlorodiphenyl silane (2.18 g, 2.06 mL, 7.91 mmol) was added, and the reaction mixture allowed to stir overnight (approx. 16 h) at rt. The mixture was then concentrated to dryness *in vacuo* and THF (10 mL) added to the crude. Triethylamine hydrochloride was removed as a white solid by filtration. The flask and precipitate were washed (3 × 5 mL) with THF, and the resulting solution concentrated to dryness *in vacuo*. Ice-cold pentane (15 mL) was added to the residue, the mixture briefly sonicated and the compound allowed to crystallise in the fridge for 2 h. The solid was collected by filtration and washed with ice-cold pentane, yielding **14** (1.77 g, 91%) as a white crystalline solid. m.p. 68-70 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.75-7.71 (m, 4H, C*H*), 7.44-7.39 (m, 6H, C*H*), 1.08 (s, 9H, *CH*), N*H* protons were not observed; ^{13}C NMR (100 MHz, CD_2Cl_2): δ 135.8, 134.1, 130.0, 128.0, 27.4, 19.4. All other spectroscopic and analytical properties were identical to those reported in the literature.[30]

3',6'-bis(Diethylamino)-2-(prop-2-yn-1-yl)spiro[isoindoline-1,9'-xanthen]-3-one (16f): To a solution of rhodamine B **22** (1 g, 2.09 mmol) in anhydrous 1,2-dichloroethane (82 mL) was added dropwise $POCl_3$ (2.08 g, 1.26 mL, 13.58 mmol) with vigorous stirring, under an inert atmosphere of argon. The mixture was stirred under reflux at 80 °C for 6 h, after which it was cooled to rt and concentrated to dryness *in vacuo*. The resulting

residue was dissolved in THF (30 mL) and treated with NEt_3 (1.21 g, 1.66 mL, 12.0 mmol), followed by propargylamine (121 mg, 0.14 mL, 2.2 mmol). The solution was stirred overnight (approx. 16 h) at rt, after which it was concentrated to dryness *in vacuo* and subjected to silica gel column chromatography (eluent: CH_2Cl_2) yielding 68% (685 mg) of desired product **16f** as a pale pink solid. R_f = 0.82 (CH_2Cl_2). m.p. 191-193 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93-7.90 (m, 1H, C*H*), 7.44-7.39 (m, 2H, C*H*), 7.10-7.08 (m, 1H, C*H*), 6.46 (d, *J* = 8.9 Hz, 2H, C*H*), 6.39 (d, *J* = 2.5 Hz, 2H, C*H*), 6.26 (dd, J_1 = 2.5 Hz, J_2 = 8.9 Hz, 2H, C*H*), 3.94 (d, *J* = 2.5 Hz, 2H, C*H*), 3.33 (q, *J* = 7.1 Hz, C*H*), 1.75 (t, *J* = 2.5 Hz, 1H, C*H*), 1.15 (t, *J* = 7.1 Hz, 12H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.4, 153.8, 153.5, 148.8, 132.6, 130.4, 129.1, 128.0, 123.8, 122.9, 107.9, 105.1, 97.8, 78.3, 70.1, 64.8, 44.4, 28.5, 12.6. All other spectroscopic and analytical properties were identical to those reported in the literature.[36]

VGVA Acetylenic Peptide (16i): Acetylenic-peptide **16i** was synthesised on 200 mg scale, following the strategy described on Scheme SI1, using the appropriate amino acids. Following synthesis of the desired sequence, 5-hexynoic acid (68.9 mg, 68 µL, 0.615 mmol) was used in place of an amino acid, and a double coupling was achieved in order to ensure a quantitative reaction. An aliquot of the resulting crude was isolated, cleaved from the resin and analyzed by RP-HPLC. The cleavage cocktail mixture used was TFA/H_2O/EDT/TIPS 94:2.5:2.5:1. The cleavage took place for 4 h, under an inert atmosphere of argon. Peptide **16i** was isolated as a white solid, soluble in a mixture of H_2O/CH_3CN 1:1 + 0.1% TFA. HPLC retention time: 29.4 min. MS (ESI-HRMS): Found 1186.6520 $[M+H]^+$, 593.8314 $[M+2H]^{2+}$, which resolved to 1185.6456 ± 0.0053 [M], $C_{54}H_{87}N_{15}O_{13}S$ requires = 1185.6328. Additional synthetic and analytical details are described in the supporting information.

$(AATT)_3$ Lys Acetylenic PNA (16j): Acetylenic PNA dodecamer **16j** was synthesised on 250 mg scale, following the strategy described on Scheme SI1, using the appropriate amino acid (lysine) and PNA monomers. Following synthesis of the desired sequence, 5-hexynoic acid (68.9 mg, 68 µL, 0.615 mmol) was used in place of a PNA monomer, and a double coupling was achieved in order to ensure a quantitative reaction. An aliquot of the resulting crude was isolated, cleaved from the resin. The Cbz-protecting groups were deprotected. Resulting deprotected PNA **16j** was isolated as a white solid, soluble in H_2O. HPLC retention time: 11.4 min. MS (MALDI-MS): Found 3487.5 $[M+H]^+$, $C_{144}H_{184}N_{69}O_{38}$ requires = 3487.46; Found 3509.5 $[M+Na]^+$, $C_{144}H_{183}N_{69}NaO_{38}$ requires = 3509.44; Found 3525.4 $[M+K]^+$, $C_{144}H_{183}N_{69}KO_{38}$ requires = 3525.42. Additional synthetic and analytical details are described in the supporting information.

General Experimental Procedure for the Preparation of 3,5-disubstituted Isoxazoles (17) – Method A:[21] To a solution of *O*-silylated hydroxamic acid **11** (1 eq) in anhydrous CH_2Cl_2 was added NEt_3 (3 eq) under an inert atmosphere of argon. This was followed by the dropwise addition of a 1M solution of Tf_2O in CH_2Cl_2 (1.1 eq) at -40 °C. Once the addition was completed, the reaction mixture was allowed to stir for 1 h at 0 °C, after which alkyne **16** (8 eq) was added, and the resulting mixture stirred overnight (approx. 16 h) at rt. Next, the solution was washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness *in vacuo*.[3] The residue was purified by silica gel column chromatography to afford desired isoxazole **17**. With this procedure, we prepared cycloadducts **17a-d**.

General Experimental Procedure for the Preparation of 3,5-disubstituted Isoxazoles (17) – Method B: To a solution of *O*-silylated hydroxamic acid **11** (1 eq) in anhydrous CH_2Cl_2 was added NEt_3 (3 eq) under an inert atmosphere of argon. This was followed by the dropwise addition of a 1M solution of Tf_2O in CH_2Cl_2 (1.1 eq) at -40 °C. Once the addition was completed, the reaction mixture was allowed to stir for 3 min at -40 °C, after which it was cannulated batchwise (every 15 min over 45 min) at that temperature, onto neat alkyne **16** kept at rt (8 eq). The resulting mixture was stirred overnight (approx. 16 h) at rt. Next, the solution was washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography to afford desired isoxazole **17**. With this procedure, we prepared cycloadducts **17a-n**.

5-Hexyl-3-phenylisoxazole (17a): *Synthesis according to Method A:* Yield 49% (23 mg) of desired product **17a** as a viscous light-yellow oil. *Synthesis according to Method B:* Yield 75% (35 mg, viscous light-yellow oil). R_f = 0.58 (CH_2Cl_2). ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.79-7.76 (m, 2H, C*H*), 7.44-7.42 (m, 3H, C*H*), 6.31 (s, 1H, C*H*), 2.77 (m, 2H, C*H*), 1.74-1.68 (m, 2H, C*H*), 1.40-1.29 (m, 6H, C*H*), 0.89 (t, *J* = 7.1 Hz, 3H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, CD_2Cl_2): δ 174.9, 162.6, 130.1, 129.9, 129.2, 127.1, 99.1, 31.9, 29.2, 27.9, 27.2, 22.9, 14.2; MS (ESI-HRMS): Found 230.1541 $[M+H]^+$, $C_{15}H_{20}NO$ requires = 230.1539. All other spectroscopic and analytical properties were identical to those reported in the literature.[34a]

3,5-Diphenylisoxazole (17b): *Synthesis according to Method A:* Yield 54% (16 mg) of desired product **17b** as a white solid. *Synthesis according to Method B:* Yield 78% (23 mg, white solid). R_f = 0.58 (CH_2Cl_2). m.p. 137-139 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.85-7.83 (m, 4H, C*H*), 7.50-7.47 (m, 6H, C*H*), 6.88 (s, 1H, C*H*); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 170.7, 163.3, 130.6, 130.4, 129.6, 129.4, 129.3, 127.8, 127.1, 126.1, 97.9. All other spectroscopic and analytical properties were identical to those reported in the literature.[20c]

3-Phenyl-5-(4-(trifluoromethyl)phenyl)isoxazole (17c): *Synthesis according to Method A:* Yield 64% (37 mg) of desired product **17c** as a white crystalline solid. *Synthesis according to Method B:* Yield 78% (30 mg, white crystalline solid). R_f = 0.63 (CH_2Cl_2). m.p. 172-175 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.97 (d, *J* = 8.7 Hz, 2H, C*H*), 7.88-7.85 (m, 2H, *CH*), 7.76 (d, *J* = 8.7 Hz, 2H, C*H*), 7.50-7.48 (m, 3H, C*H*), 6.99 (s, 1H, C*H*); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 169.2, 163.5, 131.1, 130.6, 129.4, 129.2, 127.2, 126.6, 126.5, 126.4, 126.3, 99.5. All other spectroscopic and analytical properties were identical to those reported in the literature.[12c]

***N,N*-Dimethyl-4-(3-phenylisoxazol-5-yl)aniline (17d):**
Synthesis according to Method A: Yield 65% (34 mg) of desired product **17d** as a as a pale yellow solid. *Synthesis according to Method B:* Yield 57% (20 mg, pale yellow solid). R_f = 0.52 (CH_2Cl_2). m.p. 155-158 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.86-7.83 (m, 2H, *CH*), 7.68 (d, *J* = 8.9 Hz, 2H, C*H*), 7.47-7.45 (m, 3H, C*H*), 6.75 (d, *J* = 8.9 Hz, 2H, C*H*), 6.64 (s, 1H, C*H*), 3.01 (s, 6H, C*H*); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 171.6, 163.1, 152.0, 130.1, 130.0, 129.2, 127.3, 127.1, 115.4, 112.1, 94.9, 40.3; IR (cm^{-1}): ν 689.2, 771.9, 814.4, 926.1, 949.1, 1169.9, 1198.7, 1230.9, 1326.0, 1368.4, 1397.0, 1413.4, 1446.2, 1466.0, 1507.6, 1524.4, 1578.2, 1614.1, 2922.8; MS (ESI-HRMS): Found 265.1334 $[M+H]^+$, $C_{17}H_{17}N_2O$ requires = 265.1335.

3-Phenyl-5-(trimethylsilyl)isoxazole (17e), synthesis according to Method B: Yield 69% (20 mg) of desired product **17e** as a pale yellow oil. R_f = 0.57 (CH_2Cl_2). ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.82-7.79 (m, 2H, *CH*), 7.47-7.40 (m, 3H, C*H*), 6.78 (s, 1H, C*H*), 0.37 (s, 9H, C*H*); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 179.3, 161.1, 130.1, 129.7, 129.3, 127.3, 111.1, -1.83; IR (cm^{-1}): ν 505.8, 631.1, 684.6, 691.2, 760.2, 839.1, 897.5, 949.4, 971.7, 1026.5, 1056.2, 1077.9, 1097.9, 1252.2, 1385.1, 1424.1, 1458.9, 1505.2, 1548.8, 2959.9; MS (ESI-HRMS): Found 218.0995 $[M+H]^+$, $C_{12}H_{16}NOSi$ requires = 218.0996.

3',6'-bis(Diethylamino)-2-((3-phenylisoxazol-5-yl)methyl)spiro[isoindoline-1,9'-xanthen]-3-one (17f), synthesis according to Method B: Yield 66% (53 mg) of desired product **17f** as a pale pink solid. R_f = 0.17 (CH_2Cl_2/MeOH 9:1). m.p. 215-217 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.92-7.89 (m, 1H, C*H*), 7.55-7.53 (m, 2H, C*H*), 7.49-7.47 (m, 2H, C*H*), 7.36-7.35 (m, 3H, C*H*), 7.07-7.06 (m, 1H, C*H*), 6.31-6.29 (m, 4H, C*H*), 6.14 (m, 2H, C*H*), 5.80 (s, 1H, C*H*), 4.41 (s, 2H, C*H*), 3.20 (q,

J = 7.1 Hz, 8H, C*H*), 1.03 (t, *J* = 7.1 Hz, 12H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR could not be well acquired due to the closed-opened equilibrium of rhodamine's core, which hampered the ^{13}C NMR recording of a pure isomer;[37] IR (cm^{-1}): ν 699.6, 760, 787.3, 818.1, 920.3, 950, 1018.7, 1089.3, 1118.5, 1152.2, 1219.4, 1265.3, 1305.1, 1329.3, 1357.3, 1376, 1424.5, 1443.2, 1467.6, 1514.8, 1547.9, 1614.6, 1634, 1698.3, 2928.5, 2970; MS (ESI-HRMS): Found 599.3008 $[M+H]^+$, $C_{38}H_{39}N_4O_3$ requires = 599.3017; Found 300.1552 $[M+2H]^{2+}$, $C_{38}H_{40}N_4O_3$ requires = 300.1545; UV-Vis (CH_2Cl_2, rt): λ_{max} [nm] (ε [M^{-1} cm^{-1}]): 240 (2.1×10^7), 275 (9×10^6), 316 (3.7×10^6).

3-(Anthracen-9-yl)-5-(trimethylsilyl)isoxazole (17g), synthesis according to Method B: Yield 71% (30 mg) of desired product **17g** as a viscous yellow oil. R_f = 0.51 (CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H, C*H*), 8.05 (m, 2H, C*H*), 7.80 (m, 2H, C*H*), 7.51-7.43 (m, 4H, C*H*), 6.69 (s, 1H, C*H*), 0.48 (s, 9H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.6, 159.1, 131.3, 130.8, 128.7, 128.6, 126.4, 125.9, 125.5, 123.7, 116.2, -1.5; IR (cm^{-1}): ν 622.5, 734.8, 759.4, 844.8, 1073.0, 1252.8, 1307.2, 1381.3, 1617.4, 1637.8, 2850.3, 2922.9, 2959.2, 3050.2, 3411.5, 3476.2, 3554.9; MS (APCI-HR-MS): Found 318.1313 $[M+H]^+$, $C_{20}H_{20}NOSi$ requires = 318.1314.

3-(Naphthalen-1-yl)-5-(trimethylsilyl)isoxazole (17h), synthesis according to Method B: Yield 67% (24 mg) of desired product **17h** as a viscous colorless oil. R_f = 0.49 (CH_2Cl_2). ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.37-8.35 (m, 1H, C*H*), 7.96-7.91 (m, 2H, C*H*), 7.70-7.67 (m, 1H, C*H*), 7.56-7.52 (m, 3H, C*H*), 6.77 (s, 1H, C*H*), 0.42 (s, 9H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, CD_2Cl_2): δ 178.4, 161.0, 134.2, 131.5, 130.2, 128.8, 128.1, 127.6, 127.3, 126.6, 126.1, 125.6, 114.5, -1.76; IR (cm^{-1}): ν 535.0, 561.6, 630.9, 658.8, 703.3, 759.8, 773.9, 798.9, 840.2, 887.1, 934.6, 971.8, 1026.7, 1076.0, 1130.3, 1180.4, 1214.7, 1252.3, 1325.2, 1358.4, 1371.1, 1395.4, 1512.9, 1579.5, 1597.5, 1730.4, 2900.2, 2959.1, 3050.1; MS (ESI-HRMS): Found 268.1153 $[M+H]^+$, $C_{16}H_{18}NOSi$ requires = 268.1152.

3-(Naphthalen-1-yl)-5-phenylisoxazole (17i), synthesis according to Method B: Yield 72% (26 mg) of desired product **17i** as a white solid. R_f = 0.53 (CH_2Cl_2). m.p. 108-110 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.45-8.42 (m, 1H, C*H*), 7.98 (m, 1H, C*H*), 7.93 (m, 1H, C*H*), 7.88 (m, 2H, C*H*), 7.75 (m, 1H, C*H*), 7.59-7.46 (m, 6H, C*H*), 6.88 (s, 1H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, CD_2Cl_2): δ 170.1, 163.5, 134.3, 131.4, 130.7, 130.6, 129.5, 128.9, 128.1, 127.8, 127.4, 127.3, 126.7, 126.2, 126.0, 125.6, 101.5; IR (cm^{-1}): ν 535.5, 565.7, 653.8, 668.1, 687.9, 763.1, 773.7, 797.9, 864.6, 915.7, 936.0, 948.1, 970.7, 1026.4, 1098.1, 1130.6, 1143.5, 1162.7, 1180.6, 1214.2, 1261.7, 1292.2, 1335.2, 1364.7, 1378.6, 1408.4, 1446.6, 1475.4, 1497.3, 1514.5, 1571.5, 1590.6, 1612.4, 1730.7, 1818.8, 1951.1, 2850.8, 2921.6, 3051.9, 3126.1; MS (ESI-HRMS): Found 272.1068 $[M+H]^+$, $C_{19}H_{14}NO$ requires = 272.1070.

***N,N*-Dimethyl-4-(3-(naphthalen-1-yl)isoxazol-5-yl)aniline (17j), synthesis according to Method B:** Yield 61% (25.5 mg) of desired product **17j** as a pale yellow solid. R_f = 0.44 (CH_2Cl_2). m.p. 122-125 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.47-8.45 (m, 1H, C*H*), 7.97-7.92 (m, 2H, C*H*), 7.75-7.71 (m, 3H, C*H*), 7.58-7.53 (m, 3H, C*H*), 6.77 (m, 2H, C*H*), 6.65 (s, 1H, C*H*), 3.02 (s, 6H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, CD_2Cl_2): δ 170.9, 163.3; 152.0, 134.2, 131.5, 130.3, 128.8, 127.9, 127.8, 127.4, 127.2, 126.6, 126.2, 125.6, 115.4, 112.2, 98.5, 40.3; IR (cm^{-1}): ν 659.7, 776.3, 785.4, 803.3, 819.5, 935.7, 949.9, 1063.1, 1123.3, 1169.0, 1195.8, 1226.9, 1263.7, 1362.5, 1408.4, 1441.8, 1480.7, 1518.3, 1609.6, 2894.5; MS (ESI-HRMS): Found 315.1491 $[M+H]^+$, $C_{21}H_{19}N_2O$ requires = 315.1492.

1,4-bis(5-(Trimethylsilyl)isoxazol-3-yl)benzene (17k), synthesis according to Method B: Yield 50% (10.6 mg; 71% per function) of desired product **17k** as a viscous colorless oil. R_f = 0.54 (CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 4H, C*H*), 6.82 (s, 2H, C*H*), 0.37 (s, 18H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.6, 160.5, 130.9, 127.8, 111.1, -1.85; IR (cm^{-1}): ν 514.2, 529.8, 608.0, 634.6, 691.7, 706.1, 762.8, 798.1, 822.8, 841.5, 897.5, 947.7, 971.1, 1021.5, 1063.7, 1101.8, 1217.6, 1251.9, 1276.0, 1352.4, 1373.6, 1410.6, 1447.2, 1523.0, 1559.4, 2854.1, 2924.8, 2960.4, 3122.6; MS (ESI-HRMS): Found 357.1451 $[M+H]^+$, $C_{18}H_{25}N_2O_2Si_2$ requires = 357.1449.

3,3'-(5'-(4-(5-(Trimethylsilyl)isoxazol-3-yl)phenyl)-[1,1':3',1''-terphenyl]-4,4''-diyl)bis(5-(trimethylsilyl)isoxazole) (17l), synthesis according to Method B: Yield 39% (38 mg; 73% per functionality) of desired product **17l** as a white solid. R_f = 0.38 (CH_2Cl_2). m.p. 42-44 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.6 Hz, 6H, C*H*), 7.93 (s, 3H, C*H*), 7.85 (d, *J* = 8.6 Hz, 6H, C*H*), 6.84 (s, 3H, C*H*), 0.39 (s, 27H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.5, 160.7, 142.4, 142.1, 129.1, 128.2, 127.9, 125.7, 111.1, -1.79; IR (cm^{-1}): ν 505.6, 535.0, 575.2, 609.3, 630.5, 672.2, 702.0, 736.9, 758.5, 804.9, 836.5, 898.8, 949.7, 971.6, 1018.3, 1050.3, 1098.9, 1209.0, 1251.8, 1303.2, 1377.2, 1403.2, 1417.9, 1449.6, 1516.8, 1545.7, 1571.5, 1596.0, 1611.4, 1918.0, 2899.7, 2958.7, 3050.3; MS (ESI-HRMS): Found 724.2832 $[M+H]^+$, $C_{42}H_{46}N_3O_3Si_3$ requires = 724.2841; Found 362.6461 $[M+2H]^{2+}$, $C_{42}H_{47}N_3O_3Si_3$ requires = 362.6457.

3,4,5-Triphenylisoxazole (17m), synthesis according to Method B: Desired derivative **17m** was isolated by silica gel column chromatography (eluent: CH_2Cl_2), along with traces of an unidentified by-product. Further purification was attempted by means of preparative TLC plates (3 ×; eluent: CH_2Cl_2). However, **17m** could not be isolated pure. 6 mg of that mixture was obtained as a white solid, thus affording less than 15% yield in **17m**. R_f = 0.69 (CH_2Cl_2). ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.15 (m, C*H*), 7.52-7.44 (m, C*H*); MS (ESI-HRMS): Found 298.1225 $[M+H]^+$, $C_{21}H_{16}NO$ requires = 298.1226.

5-(4-Ethynylphenyl)-3-phenylisoxazole (17n), synthesis according to Method B: Yield 18% (7 mg) of product **17n** as a white solid. R_f = 0.58 (CH_2Cl_2). m.p. 96-98 °C; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.86-7.84 (m, 2H, C*H*), 7.80 (d, *J* = 8.6 Hz, 2H, C*H*), 7.60 (d, *J* = 8.6 Hz, 2H, C*H*), 7.49-7.47 (m, 3H, C*H*), 6.90 (s, 1H, C*H*), 3.26 (s, 1H, C*H*); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 169.8, 163.4, 133.1, 130.5, 129.3, 129.1, 127.9, 127.1, 126.0, 124.3, 98.7, 83.2, 79.5; IR (cm^{-1}): ν 511.4, 543.2, 615.2, 627.1, 663.7, 692.7, 764.0, 819.0, 845.3, 916.6, 949.9, 1044.9, 1090.1, 1112.1, 1399.2, 1413.7, 1462.4, 1493.7, 2920.0, 3298.2; MS (ESI-HRMS): Found 246.0914 $[M+H]^+$, $C_{17}H_{12}NO$ requires = 246.0913.

VGVA Peptide-isoxazole conjugate (17o): To a solution of *O*-silylated hydroxamic acid of phenyl **11a** (180 mg, 0.48 mmol) in anhydrous CH_2Cl_2 (7.2 mL) was added NEt_3 (146 mg, 202 µL, 1.44 mmol) under an inert atmosphere of argon. This was followed by the dropwise addition of a 1 M solution of Tf_2O in CH_2Cl_2 (528 µL, 0.528 mmol) at -40 °C. Once the addition was completed, the reaction mixture was allowed to stir for 3 min at -40 °C, after which it was added to a suspension of resin-bound ethynyl-functionalized peptide **16i** (100 mg, 0.048 mmol) in DMF (2 mL). The resulting mixture was shaken overnight (approx. 16 h) at 50 °C. The solution was then filtered and washed with DMF (5 × 5 mL) and CH_2Cl_2 (7 × 5 mL). The resulting crude was isolated, cleaved from the resin, analyzed and purified by RP-HPLC. The cleavage cocktail mixture used was TFA/H_2O/EDT/TIPS 94:2.5:2.5:1. The cleavage took place for 4 h, under an inert atmosphere of argon. Peptide **17o** was isolated as a white solid, soluble in a mixture of H_2O/CH_3CN 1:1 + 0.1% TFA. HPLC retention time: 26.7 min. MS (MALDI-HRMS): Found 1305.6781 $[M+H]^+$, $C_{61}H_{93}N_{16}O_{14}S$ requires = 1305.6778; Found 1327.7 $[M+Na]^+$, $C_{61}H_{92}N_{16}NaO_{14}S$ requires = 1327.66; Found 1343.6 $[M+K]^+$, $C_{61}H_{92}N_{16}KO_{14}S$ requires = 1343.63. Based on the analytical RP-HPLC chromatogram, it was calculated that 49% conversion was obtained, of which 41% corresponded to desired cycloadded peptide **17o**, and 8% to unidentified subproducts. 51% of starting acetylenic-peptide **16i** were recovered thereby resulting in 84%

yield of peptide-isoxazole conjugate. Percentages are given as area%. Additional synthetic and analytical details are described in the supporting information.

$(AATT)_3$ Lys PNA-isoxazole conjugate (17p): To a solution of *O*-silylated hydroxamic acid of phenyl **11a** (19 mg, 0.040 mmol) in anhydrous CH_2Cl_2 (50 µL) was added NEt_3 (12.2 mg, 16.6 µL, 0.12 mmol) under an inert atmosphere of argon. This was followed by the dropwise addition of a 1 M solution of Tf_2O in CH_2Cl_2 (44 µL, 0.044 mmol) at -40 °C. Once the addition was completed, the reaction mixture was allowed to stir for 3 min at -40 °C, after which it was added to a suspension of resin-bound Cbz-protected ethynyl-functionalized PNA **16j** (10 mg, 0.0041 mmol) in DMF (200 µL). The resulting mixture was shaken overnight (approx. 16 h) at 50 °C. The solution was then filtered and washed with DMF (5 × 5 mL) and CH_2Cl_2 (7 × 5 mL). The resulting crude was isolated, cleaved from the resin and the Cbz groups deprotected. Resulting deprotected PNA **17p** was analyzed and purified. PNA **17p** was isolated as a white solid, soluble in a mixture of H_2O/CH_3CN 1:1 + 0.1% TFA. HPLC retention time: 18.1 min. MS (MALDI-HRMS): Found 3606.6648 $[M+H]^+$, $C_{151}H_{189}N_{70}O_{39}$ requires = 3606.4952; Found 3628.6714 $[M+Na]^+$, $C_{151}H_{188}N_{70}NaO_{39}$ requires = 3628.4772; Found 3644.6257 $[M+K]^+$, $C_{151}H_{188}N_{70}KO_{39}$ requires = 3644.4511. **17p** was formed in 43% (%area of desired cycloadduct in the RP-HPLC chromatogram). Additional synthetic and analytical details are described in the supporting information.

5-Hexyl-3-phenyl-4,5-dihydroisoxazole (19): To a solution of *O*-silylated hydroxamic acid of phenyl **11a** (75 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (3 mL) was added NEt_3 (60.7 mg, 83.6 µL, 0.6 mmol) under an inert atmosphere of argon. This was followed by the dropwise addition of a 1M solution of Tf_2O in CH_2Cl_2 (220 µL, 0.22 mmol) at -40 °C. Once the addition was completed, the reaction mixture was allowed to stir for 1 h at 0 °C, after which 1-octene **18** (179.6 mg, 252 µL, 1.6 mmol) was added, and the resulting mixture stirred overnight (approx. 16 h) at rt. Next, the solution was washed with H_2O (2 × 1 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2), yielding **19** (36.6 mg, 79%) as a white solid. R_f = 0.56 (CH_2Cl_2). m.p. 49-52 °C; ^{1}H NMR (400 MHz, CD_2Cl_2, *denotes rotamer peaks): δ 7.65-7.62 (m, 2H, C*H*), 7.39-7.37 (m, 3H, C*H*), 4.67-4.65 (m, 1H, C*H*), 3.40-3.33 & 2.97-2.90* (m, 2H, C*H*), 1.78-1.70 & 1.62-1.55* (m, 2H, C*H*), 1.47-1.24 (m, 8H, C*H*), 0.89-0.85 (t, *J* = 7.0 Hz, 3H, C*H*), the proton assignment has been done by 2D analysis; ^{13}C NMR (100 MHz, CD_2Cl_2): δ 156.7, 130.5, 130.1, 129.0, 126.8, 81.9, 40.2, 35.7, 32.1, 29.5, 25.9, 22.9, 14.2. All other spectroscopic and analytical properties were identical to those reported in the literature.[20c]

(4-(Methoxycarbonyl)phenyl)boronic acid (23): To a solution of 4-carboxyphenyl boronic acid **24** (1 g, 6.03 mmol) in anhydrous MeOH (17.4 g, 22 mL, 543 mmol) was slowly added $SOCl_2$ (4.5 g, 2.7 mL, 37.39 mmol) under an inert atmosphere of argon. The resulting mixture was stirred for 2 h 30 at 50 °C, after which it was concentrated to dryness *in vacuo*. The compound was also dried under high vacuum to remove any residual trace $SOCl_2$. The residue was dissolved in EtOAc (50 mL) and washed with brine (20 mL). The aqueous phase was extracted with EtOAc (2 × 30 mL) and the combined organic phases dried over anhydrous $MgSO_4$, filtered and concentrated to dryness *in vacuo* to yield **23** as a white solid (1 g, 92%). No further purification was required. m.p. 232-233 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, *J* = 8.0 Hz, 2H, C*H*), 8.17 (d, *J* = 8.0 Hz, 2H, C*H*), 3.98 (s, 3H, C*H*), BO*H* proton signals were not observed; ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1, 135.7, 134.0, 133.6, 129.1, 52.5. All other spectroscopic and analytical properties were identical to those reported in the literature.[39, 47]

5'-(4-Carboxyphenyl)-[1,1':3',1''-terphenyl]-4,4''-dicarboxylic acid (26): Tri-ester **27** (370 mg, 0.77 mmol) was dissolved in a mixture of THF/MeOH 1:1 (14 mL), and 7 mL of 4.8 M aq. solution of NaOH (1.344 g, 33.6 mmol) added at 0 °C. The reaction mixture was stirred overnight (approx. 16 h) at rt, after which the solvents were evaporated *in vacuo*. The residue was solubilised in H_2O (minimum amount, *i.e.* 7 mL) and the resulting solution acidified to pH ~2 with 1 M aq. solution of HCl. The resulting white precipitate was filtered, collected, resuspended in H_2O (5 mL), sonicated and filtered once more. Through this procedure, NaCl salts were washed out. The white solid was collected and dried under high vacuum (233 mg, 70% yield in **26**). m.p. 316-319 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (s, 3H, C*H*), 8.06 (s, 12H, C*H*), COO*H* proton signals were not observed; ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.1, 143.8, 140.8, 130.0, 129.9, 127.4, 125.6. All other spectroscopic and analytical properties were identical to those reported in the literature.[41, 48]

Dimethyl 5'-(4-(methoxycarbonyl)phenyl)-[1,1':3',1''-terphenyl]-4,4''-dicarboxylate (27): Na_2CO_3 (1.01 g, 9.53 mmol), 4-methoxycarbonylphenylboronic acid **23** (1.28 g, 7.15 mmol), 1,3,5-tribromobenzene **25** (500 mg, 1.59 mmol) and $[Pd(PPh_3)_4]$ (184 mg, 0.159 mmol) were weighed into a 10-20 mL microwave flask, under an inert atmosphere of argon. A degassed mixture of toluene/EtOH 1:1 (17 mL) was then added to the solids (Note: toluene and EtOH are degassed in two different two-neck flasks; simple argon bubbling (with two argon balloons and two thick needle outlets), for 45 min is enough to degas the solvents for this reaction; drops of water are added in EtOH). The resulting mixture was stirred for 5 min before being heated at 110 °C for 4 h under microwave conditions (the pressure rose up to 8 bars). The solution was then diluted with CH_2Cl_2 (250 mL) and extracted with H_2O (3 × 100 mL) and brine (1 × 100 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (eluent: Cy to Cy/EtOAc 4:6) yielding 48% of desired product **27** (370 mg) as a white solid. R_f = 0.45 (Cy/EtOAc 4:6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.2 Hz, 6H, C*H*), 7.86 (s, 3H, C*H*), 7.77 (d, *J* = 8.5 Hz, 6H, C*H*), 3.96 (s, 9H, C*H*).[40, 49] The next step, *i.e.* saponification, was performed on the whole batch, without further characterizations of tri-ester derivative **27**.

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Keywords: cycloaddition reactions • isoxazole • click reactions • Nitrile Oxides • biorthogonal reactions

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