Azoacetylenes for the Synthesis of Arylazotriazole Photoswitches

Patrick Pfaff, Felix Anderl, Moritz Fink, Moritz Balkenhohl and Erick M. Carreira*

Laboratorium für Organische Chemie, ETH Zürich, D-CHAB, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland

ABSTRACT: We report a modular approach towards novel arylazotriazole photoswitches and their photophysical characterization. Addition of lithiated TIPS-acetylene to aryldiazonium tetrafluoroborate salts gives a wide range of azoacetylenes, constituting an underexplored class of stable intermediates. In situ desilylation transiently leads to terminal arylazoacetylenes that undergo copper-catalyzed cycloadditions (CuAAC) with a diverse collection of organoazides. These include complex molecules derived from natural products or drugs, such as colchicine, taxol, tamiflu, and arachidonic acid. The arylazotriazoles display near-quantitative photoisomerization and long thermal Z-half-lives. Using the method, we introduce for the first time the design and synthesis of a diacetylene platform. It permits implementation of consecutive and diversity-oriented approaches linking two different conjugants to independently addressable acetylenes within a common photoswitchable azotriazole. This is showcased in the synthesis of several photoswitchable conjugates, with potential applications as photoPROTACs and biotin conjugates.

The observation of photochromism in the prototypical azobenzene has inspired the study of photoswitches in diverse research contexts, ranging from materials science to medicine. With the emergence of photopharmacology, photoswitchable agents hold the promise to directly impact human health via reversible and spatiotemporal control of drug activity potentially limiting off-tissue toxicity. Although photoswitches are widely applied in various modern settings, methods for their synthesis largely rely on traditional approaches (Scheme 1A). The development and implementation of practical, convenient synthetic methods can provide access to new photoswitches with desirable photophysical properties, enabling novel applications.

Given the success of arylazopyrazoles with near-quantitative photoisomerization and high bistability pioneered by Fuchter, we envisioned that 1,4-substituted arylazotriazoles could possess beneficial photophysical properties (Scheme 1B). For biological applications, switchable scaffolds are desirable that allow convergent coupling of complex chemical structures. HEREIN, we report a novel strategy to efficiently access arylazotriazoles in a modular approach that is compatible with introduction of highly functionalized molecules (Scheme 1B). The azotriazoles described display high bistability (days to years at rt), near-quantitative photoisomerization (E→Z: >98%, Z→E: >90%) and photostability against bleaching. We further report a diacetylene platform that enables consecutive coupling of two different complex azides to furnish photoswitchable azotriazole conjugates either in a one-pot protocol or in diversity-oriented divergent two-step procedures.

In recent years, heteroaarylazobenzenes have gained considerable attention as photoswitches. A range of these incorporating pyrazoles, imidazoles and thiophenes have been synthesized and photophysically characterized (Scheme 1A). These procedures, reported to work well in simple systems, rely on either condensation reactions, electrophilic aromatic substitution or organometal addition to aryldiazonium salts. Synthetic approaches to arylazotriazoles and their implementation in complex settings relevant to biology require development of mild synthetic methods characterized by chemoselectivity and modularity. A well-established class of reactions that meets these criteria is click chemistry. Specifically, Cu(I)-catalyzed azide-alkyne cycloadditions (Cu-

---

Scheme 1: Conventional Approaches and Present Work.

A. Traditional Approaches to Azoheteroarenes

1) Beyer–Mills condensation

2) Azo coupling

3) Hydrazine condensation

4) Organolithium addition to Ph-N₂⁺

B. This Work – Chemoselective Click Chemistry

Synthetic Method

modular synthesis

compatible with complex molecules

Photophysical Studies

bistability

photoisomerization?

robustness?

from days to years

no photobleaching

Diaacetylene Platform for Orthogonal Differential Conjugation

---
AACs) have been widely adopted, and numerous approaches are available for preparation of azides and alkynes.

In this context, we sought to develop a general strategy for synthesis of aryloazotriazole switches via CuAAC (Scheme 1B), which would proceed from a common, versatile building block. The parent terminal acetylene is the prototype of a class of compounds that is underexplored and elusive. As a reactive intermediate, it would have to be generated in situ from a masked precursor, as shown for 2. Inspired by Ferin-ga’s report, we hypothesized that aryloazocetylenes might be prepared by addition of lithiated alkyne derivatives to aryldiazonium tetrafluoroborates.

Our efforts commenced with attempts to efficiently access masked aryloazocetylenes 2. Addition of lithiated TMS-acetylene to phenylidiazonium tetrafluoroborate at -78 °C led to clean formation of phenylazo-TMS-acetylene (Scheme 1B, R = Me, Ar = Ph), which was isolated after aqueous workup. During purification of the material, however, continuous degradation of the compound was observed (see SI). We hypothesized that an increase in sterically bulky of the silyl group might lead to improved stability, enabling handling and subsequent use of the azoacetylene.

Addition of lithiated TIPS-acetylene to PhNzBF4 at -78 °C led to formation of TIPS-protected phenylazooacetylene (2a, >99%, Scheme 2A). To our delight, 2a proved to be thermally stable and was stored for about a year at room temperature without decomposition as determined by ‘H-NMR. To examine the generality of the protocol, TIPS-protected aryloazocetylenes were prepared, bearing both electron-donating (2b-d, 2g-h, 2m) and electron-withdrawing substituents (2e-f, 2i-2l, 2n) in 66–99% yield. 2,6-Disubstituted aryloazocetylenes (2d-g) were prepared (74–89%), because of the beneficial photophysical properties of the corresponding aryloazonobenzenes.

To study if these novel aryloazocetylenes are sufficiently robust for derivatization, we examined function-alization reactions on masked azoacetylenes 2 and 2l (Scheme 2B). Sonogashira coupling of p-bromoazoacetylene 2j with ethinylestradiol was conducted with (t-Bu)2Pd as catalyst, giving 2o (69% yield). Alternatively, deprotection of tert-butyl ester 2l allowed subsequent esterification with cortisone, giving 2p (57% yield, two-steps).

With a broad set of TIPS-masked aryloazocetylenes in hand, we turned our attention to development of mild conditions for in situ desilylative CuAAC, compatible with functionalized, complex conjugants. Importantly, in biological applications it would be desirable to minimize subsequent onerous manipulations, such as deprotections or oxidation state adjustments, following the click conjugation step.

The thermal lability of terminal azoacetylenes 3 and their potential for dimerization suggested conditions in which their concentration is kept low over the course of the reaction. We reasoned that slow release of 3 from the TIPS precursor would be possible by controlled delivery of fluoride. Transiently produced terminal azoacetylene 3 would then undergo rapid CuAAC (Scheme 1B). Initial attempts towards controlling supply of fluoride were based on the use of a solid-liquid interface. This involved KF/MeOH and relied on slow dissolution of KF over the course of the reaction.

Examination of the scope for these conditions, however, revealed lack of generality. Further screening led to the identification of a set of liquid-liquid biphasic conditions (THF–H2O (3:1)) with aq. KF or aq. CsF/t-Bu4NBr at either rt or
Collectively, this set of reaction conditions enabled access to a wide range of azotriazoles including electron-donating and withdrawing substituents (Scheme 3).

**Scheme 3: Desilylative CuAAC of Azoacetylenes to give Azotriazoles and Photophysical Details.**

![Scheme 3: Desilylative CuAAC of Azoacetylenes to give Azotriazoles and Photophysical Details.](image)

![Selected UV-Vis spectra of compounds 4a, 4b and 4f measured in DMSO (100 μM) irradiated at 340-530 nm.](image)

We next systematically studied the photophysical properties of N-benzyl substituted azotriazoles 4a–g (Scheme 3, for details see SI). As determined by HPLC assay, all displayed high photostationary state (PSS) Z-content (>90 %) upon irradiation at the π–π* absorption bands. A representative selection of UV-Vis spectra for 4a–b and 4f in DMSO is shown in Figure 1. When compared to parent 4a, compounds bearing electron-donating substituents, as shown for p-OMe (4b), displayed red-shifted absorption spectra. No detectable photobleaching was observed for 4a–b after several irradiation cycles (see SI).

In connection to this, we observed separation of the n–π* bands of the isomeric pair E/Z-4b. This allowed selective irradiation of the n–π* absorption of Z-4b, leading to high restoration of the E-isomer by irradiation at 530 nm (91%). This is in line with observations made by Li with phenylether derivatives of arylazopyrazoles. Switches incorporating electron-withdrawing substituents, as illustrated for 4f, elicited less efficient return to the thermodynamic ground state at 530 nm and required irradiation at 415 nm for high E-PSS-content (82%). 2,6-Disubstituted arylazotriazoles, such as 4i-k, possessed slightly reduced E/Z ratio in the PSS when compared to other analogs.

The thermal half-lives of metastable Z-isomers were then determined. Electron-rich compounds (4b–c, 4e) possessed t₁/₂ in the range of weeks at 25 °C, while parent 4a, alkylated 4d and electron deficient switches (4f–g) displayed higher stability (from 196 to 331 days at 25 °C), making all ideal for applications where high bistability is desired. Bistability was influenced by N-bound residues of azotriazoles (4, R', Scheme 3). N-aryl groups (4n–o) led to shorter Z-half-lives (11–26 h) when compared to N-benzyl substituted 4a (254 d). Other N-alkyl substituted azotriazoles such as 4m (184 d) remained in a similar range. Together, these results suggest coupling of aryloazocetylenes incorporating p-electron-donating substituents to alkyl azides for optimal photoswitching properties.

![Figure 1: Selected UV-Vis spectra of compounds 4a, 4b and 4f measured in DMSO (100 μM) irradiated at 340-530 nm.](image)
In the context of applying this approach to the synthesis of photopharmacological probes, we examined access to photoswitches embedded within functionally-rich molecules (Chart 1). We thus generated azotriazole derivatives of carbohydrate glucose (5a), antiviral tamiflu (5b), lipid arachidonic acid (5c) vitamin biotin (5d), steroid ethinylestradiol (5e), alkaloid colchicine (5f) and diterpenoid taxol (5g), which were produced in 46–88% yield. This set of complex molecules comprises functional groups such as alcohols, esters, (thio)ethers, phenols, skipped dienes, ketones, amides, and urea, demonstrating broad functional group tolerance.

Conventional conjugation approaches frequently employ amide, esters or ethers for conjugant attachment to azobenzenes. In contrast, the method described herein links the objects of study directly to arylazotriazoles, which can result in shorter topological distances with increased rigidity due to fewer attendant degrees of freedom between conjoined fragments. This holds potential for design of photoswitchable probes with amplified differential biological activity between cis- and trans-photosomers.

We showed that functionally-rich molecules can be singly introduced onto arylazotriazoles either via azide (5a–d, 5f–g) or arylacetylene (5e). By extension, this gives entry to bifunctional probes linked by photoswitchable units. We were especially interested in the design of a bis-conjugation platform that would allow streamlined assembly of conjugants using two consecutive click reactions. A common challenge for generation of photoswitchable conjugates is the requirement of two independent sites of linkage and attendant orthogonal, mutually compatible modes of reactivity on either side of photoswitchable actuators. To address this issue, we turned our attention to the development of a diacetylene platform that would allow the execution of two distinctly addressable click reactions.

We wondered whether incorporation of a terminal acetylene onto the TIPS-masked azoacetylene (Scheme 4, A) would lead to a bis-conjugation platform in which the former is intrinsically “on” while the latter, by virtue of the masking group, “off,” allowing each to be sequentially engaged using the same CuAAC reaction mode (Scheme 4). The first coupling partner (R1N) would react chemoselectively at the terminal acetylene (A→B). Following formation of the first cycloadduct, addition of fluoride and a second partner (R2N) would then furnish a fully assembled photoswitchable conjugate D (B→C→D, Scheme 4). If successful, this approach would not be burdened by additional chemical manipulations. In reducing this plan to practice and due to the beneficial photophysical properties measured for phenyl ether derivatives, a terminal acetylene unit was incorporated as a p-propargyl ether, as shown for 2q, synthesized from 4-propynloxyphenyl-diazonium tetrafluoroborate (see SI).

We applied this strategy to the generation of a photoswitchable biotin–androstanolone conjugate. Sequential reaction of 2q with azido-biotin 6a and – following addition of acq. CsF – with azido-androstanolone derivative 6b produced conjugate 7a in 69% yield in a single-pot operation. Biotin conjugates have ample applications for immobilization of protein targets on streptavidin-coated surfaces. Therefore, photoswitchable biotin-conjugates have the potential to reversibly control protein immobilization and translocalization by irradiation.

The inherent versatility of diacetylene 2q enables diversity-oriented synthesis approaches to conveniently access divergent sets of photoswitchable conjugates. For example, this is desirable in the context of photoswitchable PROTACs (photoPROTACs) in which the order of introduction of the E3 ligase ligand or protein-of-interest (POI) recruiter as part of an optimization process can be chosen at will. The first click reaction then provides a common intermediate which serves as a point of departure for subsequent introduction of a variety of conjugants (different POI or E3 ligase ligands). To illustrate this concept, reaction of 2q with azido-lenalidomide (6c) generated a lenalidomide-linked azo-acetylene intermediate (not shown) which was subsequently reacted with either JQ1-azide (6d) or azido-androstanolone (6b) under the desilylative CuAAC conditions. This gives divergent access to two photoswitchable PROTAC candidates 7b and 7c, targeting bromodomain proteins (BRD) and androgen receptor (AR), respectively.

---

**Chart 1: Azotriazoles and Complex Conjugates.**

| Compound | Structure | Yield |
|----------|-----------|-------|
| 5a | ![Structure 5a](image) | 72% |
| 5b | ![Structure 5b](image) | 85% |
| 5c | ![Structure 5c](image) | 87% |
| 5d | ![Structure 5d](image) | 88% |
| 5e | ![Structure 5e](image) | 71% |
| 5f | ![Structure 5f](image) | 66% |
| 5g | ![Structure 5g](image) | 62% |
| 5h | ![Structure 5h](image) | 56% |

a. 1-arylaazoacetylene (1.0 equiv), azide derivative (1.0 equiv), CsF (1.0 equiv), Bu4NBr (1.0 equiv), 40 °C, THF–H2O (3:1, 0.1 M).

---
In summary, we have developed a novel, modular approach towards photoswitchable azotriazoles. Their thorough characterization revealed beneficial photophysical properties such as near-quantitative photoisomerization and long thermal half-lives. The underexplored class of azoacetylenes can be easily generated by addition of lithiated TIPS-acetylene to diazonium tetrafluoroborate salts. We describe in situ desilylative CuAAC reactions between azoacetylenes and a wide range of organoazides, including examples derived from complex natural products. We introduce a diacetylene platform 2q which allows the execution of two consecutive CuAACs linking two azides via a photoswitchable azotriazole either in a one-pot fashion or in a diversity-oriented two-step procedure. The modular azotriazole photoswitches reported with N-alkyl substituents offer high and predictable bistability irrespective of the substitution pattern, making them ideal motifs for the generation of bistable photoswitchable conjugates. Given the broad applicability of CuAAC conjugation strategies this new approach will find widespread use in the growing field of photoswitches.

Funding Sources
E.M.C. is grateful to ETH Zürich for financial support. P.P. is an awardee of the Scholarship Fund of the Swiss Chemical Industry (SSCI). F.A. thanks the FWF for an Erwin Schrödinger Fellowship for post-doctoral support (Project J4461); M.B. thanks the Deutsche Forschungsgemeinschaft (DFG) for a postdoctoral fellowship.

Notes
During the final stages of this work we became aware of work by Prof. T. Li (Shanghai Jiao Tong University).

ACKNOWLEDGMENT
We are grateful to Dr. Nils Trapp and Michael Solar for X-ray crystallographic analysis, and Dr. Marc-Olivier Ebert for NMR support. Prof. Donald Hilvert (ETH Zürich) is acknowledged for access and assistance with UV-Vis instrumentation.

REFERENCES
(1) Hartley, G. S. The Cis-Form of Azobenzene. Nature 1937, 140 (3537), 281.
(2) Pianowski, Z. L. Recent Implementations of Molecular Photoswitches into Smart Materials and Biological Systems. Chem. - A Eur. J. 2019, 25 (20), 5128–5144.
(3) Tochitsky, I.; Kienzler, M. A.; Isacoff, E.; Kramer, R. H. Restoring Vision to the Blind with Chemical Photoswitches. Chem. Rev. 2018, 118 (21), 10748–10773.
(4) Velema, W. A.; Szymanski, W.; Feringa, B. L. Photopharmacology: Beyond Proof of Principle. J. Am. Chem. Soc. 2014, 136 (6), 2178–2191.
(5) Broichhagen, J.; Frank, J. A.; Trauner, D. A Roadmap to Success in Photopharmacology. Acc. Chem. Res. 2015, 48 (7), 1947–1960.
(6) Weston, C. E.; Richardson, R. D.; Haycock, P. R.; White, A. J. P.; Fuchter, M. J. Arylazopyrazoles: Azoheteroarene Photoswitches Offering Quantitative Isomerization and Long Thermal Half-Lives. J. Am. Chem. Soc 2014, 136.
(7) Calbo, J.; Weston, C. E.; White, A. J. P.; Rzepa, H. S.; Contreras-Garcia, J.; Fuchter, M. J. Tuning Azoheteroarene Photoswitch
Performance through Heteroaryl Design. J. Am. Chem. Soc. 2017, 139 (3), 1261–1274.

(8) For a report of 1,1′-azobis-1,2,3-triazole as a nitrogen-rich compounds in connection with high-energy materials, see: Li, Y.-C.; Qi, C.; Li, S.-H.; Zhang, H.-J.; Sun, C.-H.; Yu, Y.-Z.; Pang, S.-P. 1,1′-Azobis-1,2,3-Triazole: A High-Nitrogen Compound with Stable N 8 Structure and Photochromism. J. Am. Chem. Soc. 2010, 132 (35), 12172–12173.

(9) During the preparation of this manuscript a multi-step synthesis route to aryloxadiazoles appeared. It uses a strategy that proceeds through the Pd-catalyzed coupling of protected hydrazines and oxidative deprotection steps to generate azotriazoles after click cycloaddition. This requires TMSI and O.

(10) Ahmanova, A.; Ahlfeld, J.; Trauner, D.; Thornmüller, S.

(11) Zhang, Z.; He, Y.; Zhou, Y.; Yu, C.; Han, L.; Li, T. Pyrazolylazophenyl Ether-Based Photoswitches: Facile Synthesis, (Near-)Quantitative Photoconversion, Long Thermal Half-Life, Easy Functionalization, and Versatile Applications in Light-Responsive Systems. Chem. – A Eur. J. 2019, 25 (58), 13402–13410.

(12) Müller, C.; Meinig, J. C.; Luy, K.; Kraus, Y.; Heise, C.; Bingham, R.; Jansen, K. I.; Qu, X.; Bartolini, F.; Kapitein, L. C.; Akmanova, A.; Ahfeld, J.; Trauner, D.; Thom-Seshold, O. Photoswitchable Picaxitel-Based Microtubule Stabilisers Allow Optical
Control over the Microtubule Cytoskeleton. *Nat. Commun.* 2020, **11**(1), 4640.

(44) Pfaff, P.; Samarasinghe, K. T. G.; Crews, C. M.; Carreira, E. M. Reversible Spatiotemporal Control of Induced Protein Degradation by Bistable PhotoPROTACs. *ACS Cent. Sci.* 2019, **5**(10), 1682–1690.

(45) Umeda, N.; Ueno, T.; Pohlmeyer, C.; Nagano, T.; Inoue, T. A Photocleavable Rapamycin Conjugate for Spatiotemporal Control of Small GTPase Activity. *J. Am. Chem. Soc.* 2011, **133**(1), 12–14.

(46) Reynders, M.; Matsuura, B. S.; Bérouti, M.; Simoneschi, D.; Marzio, A.; Pagano, M.; Trauner, D. PHOTACs Enable Optical Control of Protein Degradation. *Sci. Adv.* 2020, **6**(8), eaay5064.

(47) We previously reported successful generation of a highly bistable photoswitchable bromodomain (BRD) targeting PROTAC (see ref 44) that was based on the well-established tetrafluoroazobenzene motif. In that study, the tetrafluoroazobenzene displayed bistability that was dependent on functionalities in p- and p’-position and the electronic pattern thereby generated: pull-pull/amide-reverse amide. As shown in Scheme 3, N-alkyl substituted azotriazole photoswitches are bistable and insensitive to the electronic nature of the substituents.

(48) Fang, D.; Zhang, Z.-Y.; Li, T. Arylazo-1,2,3-Triazoles: “Clicked” Photoswitches for Versatile Functionalization and Electronic Decoupling. *ChemRxiv. Preprint,* https://doi.org/10.26434/chemrxiv.14604168.v1.