Exhaustive catalytic ortho-alkoxylation of azobenzenes: flexible access to functionally diverse yellow-light-responsive photoswitches

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ABSTRACT: We develop the first method for catalytic, exhaustive ortho-alkoxylation of azobenzene photoswitches. Alk oxylation is known to improve the photoswitch properties that control azobenzenes' success in chemical biology or materials sciences: e.g. better completeness of both E→Z and Z→E photoisomerisations, and >100 nm red-shifting of photoresponse. Our method enables straightforward late-stage diversification of photoswitches with interesting functional handles. We showcase four applications, using it to rationally tune lipophilicity, prepare isotopic tracers for metabolism studies, install full water solubility without ionic charges, and efficiently access previously difficult mixed-substituent photoswitches. We also identified a previously unstudied mixed-substituent tetra-ortho-family, difluoro-dialkoxy-azobenzenes, whose photoresponse can outperform previous 'gold standard' tetrafluoro-, dichloro-difluoro-, and tetrachloro-azobenzenes in significant ways. We thus expect that both the scaffolds we showcase and the method we develop will impact broadly on photochemistry and photopharmacology.

INTRODUCTION

Reversible E→Z and Z→E isomerisations of azobenzenes can be effected with UV/visible light, as well as with redox reactions following photocatalysis, electrocatalysis, or X-ray illumination. The high spatiotemporal precision, non-invasiveness, and rapidity with which bulk populations of azobenzenes can be photoisomerised in both E→Z and Z→E directions has made azobenzene photoswitching a powerful tool for manipulating the physical and optical properties of solid materials, the electronic and dynamic properties of soft matter, and for controlling protein functions and downstream cascades in biochemistry, cell biology, and in adult animals.

Azobenzene photoresponse and isomerisation completeness are critical for all these applications. Alkylazobenzenes respond efficiently to light only up to ca. 500 nm. They are often best photoisomerised around 360 nm (giving photostationary states (PSSs) of ca. 20:80 E:Z), and around 450 nm (PSSs ca. 80:20 E:Z). Several azobenzene substitution patterns have been developed to tune the isomers’ spectra, and thus the photoresponsive and completion of photoisomerisations. Tetra-ortho-substitutions particularly improve photoswitch performance, without necessarily compromising function (as most azobenzene photopharmaceuticals are "azo-extension" or "azo-linker" designs where only the 4,4' positions determine bioactivity). Hecht's tetra-ortho-fluoropattern gives excellent E→Z isomerisation at 500 nm and Z→E at 400 nm. Woolley's sterically congested tetra-ortho-methoxy, thiaoalkyl, -chlooro azobenzenes improved on older tetra-ortho-alkyl azobenzenes, with still better E→Z isomerisation at 550 nm (for good biocompatibility and depth penetration) making them alluring for photopharmacology in vivo; Z→E isomerisation is most complete at 400 nm (see Supporting Note 1). However, most tetra-ortho syntheses are inflexible, needing ortho-substituents to be introduced early and carried through synthesis; the only exhaustive late-stage ortho-derivatisation is tetra-ortho-chlorination (Fig 1a).

Figure 1. Towards exhaustive ortho-alkoxylation. (a) Trauner's Pd-catalysed per-ortho-chlorination. (b) Sun's Pd-catalysed mono-ortho-alkoxylation. (c) Pd-catalysed per-ortho-alkoxylation reported here.

Supporting Note 1
The particular allure of the tetra-ortho-alkoxy pattern is that, unlike tetra-halides, it offers four ideal sites for functional diversification (as the alkyl part should not affect photoresponse). Yet, lacking a modular tetra-ortho-alkoxylation method, essentially no scope of functional ortho-substituents has been explored: e.g., these positions have never been exploited for solubilisation, isotopic labelling, or rational logD tuning. Continuing our interest in polyalkylated photothepharmaceuticals,9,25,50 but aiming to avoid polyphenol alkylations (see Supporting Note 1), we here aimed to develop a flexible, late-stage, exhaustive ortho-alkoxylation procedure for azobenzenes. However, we observed rapid formation of palladium black during heating, as expected for reduction of Pd(OAc)₂ by methanol,31 likely blocking reaction progress. As reduction was slower at lower temperatures, we screened down to 40°C under strict temperature control, increasing the tetra-substitution yield to 28% (Fig 2, entries 3-4). This is unusual for CH activations, that are generally favoured by high temperatures. As nitrogen ligands can stabilise Pd(OAc)₂ against reduction, we tested pyridine and 1,1'-bipyridine; while these prevented formation of palladium black, they also blocked alkoxylation (Table S1, entries 8-10).

**Cosolvent, oxidant, additives, and catalyst loading**

We next screened cosolvents to methanol (Table S1, entries 11-25); toluene improved yields, with good results at 1 : 1 MeOH:PhMe (Fig 2, entry 5). We also screened diluted conditions, since the solubility of PIDA is only moderate: but these lowered isolated yields greatly (Table S1, entry 26), coherent with the role of a dimeric intermediate (Scheme S2). PIDA was however superior to PIFA, oxone, or K₂S₂O₈ as the oxidant, and increasing its loading (10 eq.) only reduced yields (Table S1, entries 27-32). This is coherent with expectations that excess or stronger oxidants drive parasitic oxidations of the increasingly electron-rich intermediates/product. Acidic, basic, or dehydrating additives also did not give improvements (Table S1, entries 33-40). Instead, reaction yields improved when Pd(OAc)₂ loading was raised (Fig 2, entries 6-7).

Taken together, this screening had improved isolated yields of tetra-ortho-methoxylation to reach nearly 40% (Fig 2, entry 7), corresponding to ca. 75% yield per CH activation/C·O formation, which matches pleasingly to the single-step yields of Sun.28

**Scope for azobenzene substituents**

To study this method’s substrate preference, we applied the conditions optimised for unsubstituted 1a to a set of mono-substituted azobenzenes (Fig 3). Electron-donating groups in para gave poor (alkyl 2b) to negligible (oxyether) yields, but were better tolerated in meta (2c-2d) (discussed at Scheme S2). Electron-poor parasubstituents were successful (ester 2e, halogens 2f-2g, nitro 2h). These patterns, which offer flexible derivatisation by reduction/acylation (nitro), esterification/amidation (ester), or cross-coupling (halide), can now be accessed by an easier and more tolerant route than previously possible with e.g. lithium base methods.

**Results**

**Catalytic tetra-ortho-methoxylation of azobenzene**

Palladium-catalysed CH-oxidation using the diazene as an ortho-directing group was developed from a stoichiometric into a catalytic, exhaustive ortho-chlorination method by Trauner,20 with N-chlorosuccinimide (NCS) acting as both oxidant and chlorine source (Fig 1a). We first tested if alkoxy analogues of NCS, e.g. N-methoxysuccinimide [NMS], could likewise function as both oxidant and alkoxy source, but saw no conversion (Fig 2, entry 1).

We then moved to alcohols as straightforward alkoxy sources, seeking tandem oxidation by another reagent to complete the turnover. As far as we know, Sun performed the only study of oxidative azobenzene ortho-alkoxylation, isolating mono-ortho-alkoxyazobenzenes in up to 77% yield by reacting a variety of non-, mono-meta-, and bis-meta-substituted azobenzenes with Pd(OAc)₂ (10 mol%), alcohol, and PhIL(OAc)₂ [PIDA] oxidant (1-2 eq.) at 80°C (Fig 1b, mechanism in Scheme S2).24 However, despite superstoichiometric oxidant, no polyalkoxylation was reported. Clean oxidative peri-alkoxylation is anyway a challenging prospect, as each alkoxylation step will increase the substrate’s electron density, so raising the chances of non-regioselective or undesired oxidations (Scheme S2).

Aiming to achieve up to four CH-oxidations per azobenzene for clean per-ortho-alkoxylation (Fig 1c), we began our quest using Sun’s mono-methoxylation conditions but increasing oxidant to 5 mol. eq. (Table S1). HPLC-MS analysis revealed a low but encouraging 6% yield (Fig 2, entry 2). Residual substrate and monomethoxy intermediate were the major impurities isolated, with di- and trimethoxylated intermediates as trace products only, matching the expectation of increasing reactivity per alkoxylation step.

**Figure 2.** Initial optimisation for temperature, cosolvent, and catalyst loading [NMS: N-methoxysuccinimide].

<table>
<thead>
<tr>
<th>entry</th>
<th>T [°C]</th>
<th>solvent / OMe eq.</th>
<th>Pd(OAc)₂ [eq.]</th>
<th>isol. yield [%]</th>
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<td>-</td>
</tr>
<tr>
<td>2</td>
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<td>MeOH</td>
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<td>5</td>
<td>40</td>
<td>MeOH:PhMe 1:1</td>
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<td>7</td>
<td>40</td>
<td>MeOH:PhMe 1:1</td>
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**Figure 3.** Substituent effects on tetra-methoxylation [isolated yields].
**ortho-alkoxylation as a functional handle**

To explore the scope for exhaustive alkylation to products other than tetramethoxy species, we tested alcohols other than methanol, and performed reactions on partially-ortho-substituted substrates (Fig 4). Secondary alcohols (iPrOH) gave only traces of tetraalkylation, and tertiaro alcohols (BuOH) gave no conversion, which is understandable due to steric. Though electron-poor primary alcohols (CF₃CH₂OH) were unreactive, we found good scope for primary alcohols, which enables tackling four novel applications:

(1) **Lipophilicity** is a key property for cellular pharmacology, impacting apparent on-target affinity, off-target binding, subcellular localisation, bioavailability, and membrane permeation. Lipophilicity Tuning

<table>
<thead>
<tr>
<th>Lipophilicity Tuning</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
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<tr>
<td>3a</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>(OEt)</td>
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<tr>
<td>3b</td>
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<td>(OMe)</td>
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<tr>
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<td>(OMe)</td>
<td>(OMe)</td>
<td>OEt</td>
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<tr>
<td>3d</td>
<td>(OMe)</td>
<td>OEt</td>
<td>OEt</td>
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<tr>
<td>3e</td>
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<td>OEt</td>
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<td>3f</td>
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**Mixed Substituents**

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<tbody>
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<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>(F)</td>
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<td>OMe</td>
<td>(F)</td>
<td>OMe</td>
<td>(F)</td>
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<tr>
<td>3m</td>
<td>OMe</td>
<td>OMe</td>
<td>(F)</td>
<td>(F)</td>
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**Isotopic Labelling**

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<td>OCD₃</td>
<td>OCD₃</td>
<td>OCD₃</td>
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**Water Solubility**

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<th>R³</th>
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<tbody>
<tr>
<td>3o</td>
<td>OPrOH</td>
<td>OPrOH</td>
<td>OPrOH</td>
<td>OPrOH</td>
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Figure 4. Per-ortho-alkoxylation of a range of azobenzenes giving otherwise difficult to access or unreported substituent patterns. Bracketed residues, e.g. (OMe), were present in the starting materials; bold residues were introduced by the reaction [isolated yields].

(2) **Asymmetry**: The optical properties of symmetric tetra-ortho-substituted azobenzenes have been explored (see Introduction). However, only a few asymmetric tetra-ortho azobenzenes (where substituents differ from each other) have been studied, and these only recently: yet strong performance enhancements were already found. Exhaustive alkylation of partially ortho-substituted substrates offers a convenient synthetic access to otherwise time-intensive mixed-substituent patterns that have not yet been systematically explored. Pleasingly, mixed fluoro/alkoxy tetra-ortho azobenzenes 3k-3m were all accessed with moderate yields, for later study.

(3) **Metabolism**: As photopharmacology moves towards applications in adult animals, monitoring the metabolism of yellow/red-light responsive photoswitches will become increasingly urgent. We know of no reports preparing isotopically-labelled photopharmaceuticals for unbiased MS-based metabolite studies, which typically require light/heavy drug pairs with M+5 for the heavy drug, but that exhaustive ortho-alkylation is an attractive method to do so. Using CH₄OH/CD₃OD provides up to M+12 difference in light/heavy masses of scaffold-intact metabolites, M+6 difference of the principal hypothesised azobenzene metabolites (N=N scission products), or M+9 difference of mono-ortho-demethylated species: all of which are sufficient for unbiased metabolite detection, and which may reveal the in vivo fate of photopharmaceuticals. Yields of tetra-ortho-(OCD₃) derivatisation were good (3n).

(4) **Solubility**: Like most photoswitches (typically, flat aromatics), azobenzenes have very poor water solubility and tend to aggregate; this complicates their photoswitching and spectra, and hampers applications from materials sciences through to biology. Exhaustive alkylation with diols could be a late-stage solubilising method that also provides beneficial tetra-ortho photoresponse, without crowding the para-positions needed for "azo-extension" and "azo-linker" photopharmacology, and without charges that complicate bioactivity. Tetra-alkoxylation with propylene glycol gave good isolated yields of water-soluble tetrol 3o (45% per substitution): the first example we know of such solubilisation in photoswitching.

**Photoswitching performance**

The tetra-ortho-alkoxyazobenzenes’ optical properties matched Woolley’s reports. They absorb well in the visible, with substantial separation between the isomers’ band maxima giving ca. 80% Z at PSS under 550 nm, and ca. 85% E at PSS under 405 nm (Fig S1b, 3i/3o). Bulk photoswitching with blue and green light was efficient; and despite low absorption coefficients above 550 nm they can be isomerised by yellow light up to 600 nm (Fig S2a, Fig S2b). The spontaneous Z→E relaxation of all derivatives was slow on relevant timescales (half-lives > 7 days; Fig S2a). Bidirectional photoswitching was fully reversible without detectable losses over tens of cycles (Fig S2b). Water-soluble tetrol 3o was assayed in all-aqueous buffer, supporting the utility of this approach for biology (Fig 5, S2).

This straightforward access to per-ortho-alkoxyalted azobenzenes can now drive systematic investigations, identifying promising new switches. For example, difluoro-dialkoxy-azobenzenes were only accessed once before, though their photoswitching was not investigated and they have not been practically applied. We saw that both asymmetric and symmetric difluoro-dialkoxy 3m and 3l had ca. 20 nm more separation between the n-π* bands of their E- and Z-isomers than tetra-alkoxy derivatives (Fig S2c). This greater separation drives excellent completeness of E→Z photoswitching under green light (only 4.5% E remaining, Fig 5a), which outperforms tetra-ortho-alkoxy- (20%) and even difluoro-dichloro-azobenzenes (15%) by several fold, while all have comparable Z→E conversion.

Symmetric 3i also has a ca. 20 nm red-shift of E→Z photoresponse compared to popular tetra-ortho-fluoro species (asymmetric 3m has a 30 nm blue-shift); and both enjoy the benefits of the flexible alkoxy handle. This performance and access recommend them for adoption, and should more broadly motivate studies of mixed substitution patterns to refine the photochemistry, biophysical properties, and functions accessible for photopharmacology.
switches may prove particularly valuable for chemical biology compared to thrombomodulin photoswitches (ter led us to polyased metabolism studies; conversion to water-spectrally identical series of derivatives; poly-substituents before azobenzene formation. Simplifies synthesis in general by a possible otherwise alcohols.

electron.

Figure 5. Optical properties. (a-b) Photostationary state (PSS) E:Z proportions (by HPLC) and corresponding UV-Vis absorption spectra. (c) E=Z photoisomerisation timecourses illustrating variations of photoresponse speed and completeness with wavelength. E→Z photoswitching was performed with the annotated test wavelengths starting at t=1 min; at the end of each test, Z→E back-switching was performed at 435 nm or 405 nm to verify photoreversibility (LED sources, see discussion at Fig S2-S3). Spectra measured in MeCN (3i, 3m, 3l) or in deionised water (3o).

Conclusion

Tetra-ortho-alkoxazobenzenes feature all-visible-light bidirectional photoswitching that is substantially complete in both E→Z and Z→E directions. Unlike bridging or tetra-ortho-halogenation approaches to redshift azobenzene photoresponses, per-ortho-alkoxylation offers up to four flexible handles for installing functionality, potentially without compromising bioactivity or Z-isomer stability.

We here present the first method for exhaustive ortho-alkoxylation of electron-poor to electron-neutral azobenzenes with primary alcohols, giving isolated yields around 50-75% per alkoxylation. The congested structures accessible by this method are not easily accessible otherwise. It both enables late-stage diversification, and also simplifies synthesis in general by avoiding the need to install alkoxy substituents before azobenzene formation.

We also present method applications that may extensively impact photopharmacology (Fig 4): stepwise tuning of lipophilicity in a spectrally identical series of derivatives; poly-deuteration for unbiased metabolism studies; conversion to water-soluble uncharged polyols; and new access to hitherto poorly-studied switches. The latter led us to identify difluoro-dialkox-azobenzenes as highly performant photoswitches (Fig 5). Since the greater sensitivity of tetra-ortho-alkoxazobenzenes to bithiol-mediated degradation (as compared to their tetra-ortho-halo counterparts) is a concern for long-term biological studies, this method towards new difluoro-dialkox switches may prove particularly valuable for chemical biology; by finally combining the biological stability of halogenation with the functional flexibility of alkoxylation (on top of the general photophysical benefits of tetra-ortho-substitution patterns).

This straightforward and flexible method for functional photoswitch tuning can therefore promote access to a range of efficiently and bidirectionally visible-light responsive, metabolically traceable, solubilised, and rationally tunable azobenzene photoswitches, with particular applications to materials sciences and chemical biology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

PDF containing synthetic protocols; photocharacterisation; and NMR spectra.

Dataset (XLSX) with E/Z and PSS spectra of selected compounds.

AUTHOR INFORMATION

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Author Contributions

A.M.-D. performed all synthesis, characterisation, and data assembly. O.T.-S. designed the study, supervised experiments, and wrote the manuscript.

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ABBREVIATIONS
NMS: N-methoxysuccinimide; PIDA, phenylidione(III) diacetate; PIFA, phenylidione(III) bis(trifluoroacetate); PBS, phosphate-buffered saline.

REFERENCES

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