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Conversion of Aryl Azides to *O*-Alkyl Imidates via Modified Staudinger Ligation

José A. Restituyo,[†] Lindsay R. Comstock,[†] Scott G. Petersen,[‡] Thomas Stringfellow,[†] and Scott R. Rajski*,[†]

The School of Pharmacy and Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53705

srrajski@pharmacy.wisc.edu

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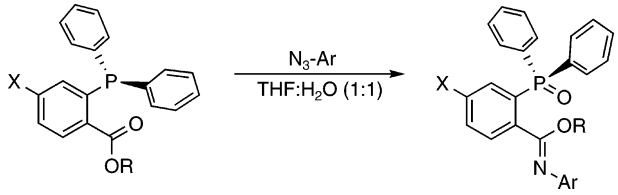
ABSTRACT



***o*-Carboalkoxy triarylphosphines are shown to react with aryl azides to provide Staudinger ligation products bearing *O*-alkyl imidate linkages. This is in contrast to alkyl azides whose ligation to *o*-carboalkoxy triarylphosphines has been reported to yield amide-linked materials. This extension of the Staudinger ligation for coupling of abiotic reagents under biocompatible conditions highlights the utility of commercially available triarylphosphines through which suitable linkers can be attached via an ester moiety.**

Small molecules orthogonal to cellular constituents and permissive of highly selective reactions with abiotic reagents have allowed tremendous advances in our understanding of biological processes. Seminal work by Bertozzi and co-workers highlights the use of cell surface-exposed ketones and azides in elucidating structural and functional roles of glycoconjugates, many of which are intimately involved in human disease.[1−8] Of particular interest is the coupling of azides with triarylphosphines of type **1** in what has been termed the Staudinger ligation.[8−10] Both ligation components are abiotic and therefore have a unique niche as biochemical reagents. Closely related ligations have been elegantly devised as powerful synthetic methods.[11−13] In developing tools by which to dissect DNA and protein methylation patterns, we sought to apply phosphines of class **1** toward the Staudinger ligation, or coupling, of aryl azides. In the course of these efforts, we observed that agents bearing the core structure **1** couple very efficiently with aryl azides to afford *O*-alkyl imidates. Such products have not been reported in Staudinger ligations of alkyl azides, nor was there evidence of such reactivity with alkyl azides in our hands.[8−10] This finding may impact the efforts of those using Staudinger ligation to understand cellular processes with small molecules. Moreover, this observed reactivity highlights the utility of commercially available triarylphosphines en route to efficient Staudinger ligation.

[†] The School of Pharmacy.
[‡] Department of Chemistry.

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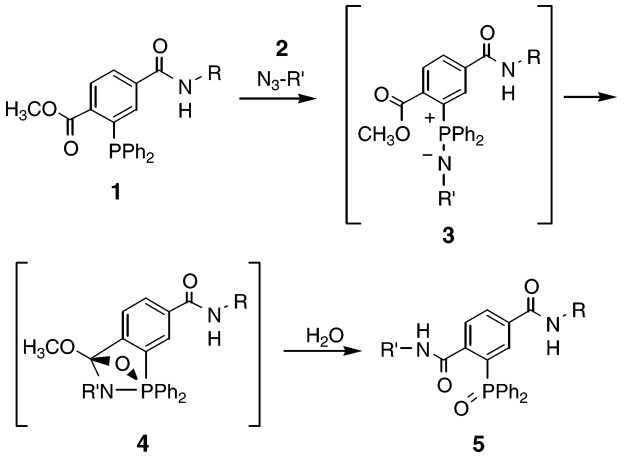
The ligation of alkyl azides to triarylphosphines of type **1** is well-known.[8−10] Bertozzi and co-workers have found that amides of type **5** are formed with yields and stabilities suitable for biochemical applications.[8−10] However, analogous couplings involving aryl azides, especially purinyl azides, were not apparent in the literature. We reasoned that similar chemistry could be applied to the identification of adenosine-linked materials bearing a C8 azide moiety. This invokes the utility of 8-azidoadenosine as a tetherable handle in addition to its more classical use as a photoaffinity label.[14,15] The proposed mechanism for triarylphosphine coupling to alkyl azides (Scheme 1) suggested that reaction

Scheme 1



of spectroscopically unique **6a**[16] with **7** (Figure 1) would afford the amide-linked adenylate **8a**.[8] Similarly, convergence of glycine-linked **6b** with **7** would afford the acylguanidine **8b**. The application of this chemistry to biological purposes mandated that the coupling reaction be performed in wet THF.[17] Aqueous reaction conditions are also needed for hydrolysis of transient phosphonium intermediate **4** to form phosphine oxide **5** (Scheme 1).[8]

Ligated material tentatively assigned as **8a** was generated in 92% yield over 2 h in 1:1 THF/H_2O with both coupling

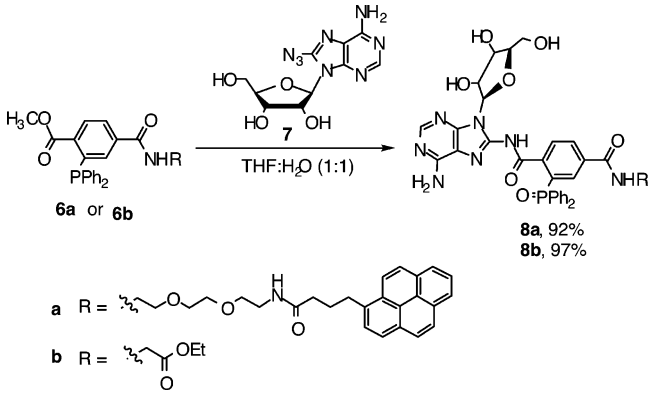


Figure 1. Primary Staudinger ligation components and anticipated adducts.[18] Yields based on HPLC integration.[19]

partners in a concentration of 1 mM at 30 °C.[19] Coupling of **6b** with **7** proceeded in an almost identical fashion, affording the presumed **8b** in 97% yield as ascertained by HPLC analysis.[18] This is in stark contrast to the same coupling with previously reported alkyl azide 5′-azido-2′,3′-isopropylideneadenosine;[19] complete consumption of this alkyl azide requires ∼24 h.[19] Importantly, control reactions of **6a** with adenosine afforded no change to either structure as evaluated by RP-HPLC, thus verifying the azide requirement.[14] Involvement of the free 5′ alcohol on **7** was ruled out via TIPS protection of this moiety and subsequent RP-HPLC analysis of silyl ether ligation to **6a** and **6b**; 5′-silylation does not impact the reaction rate or product profile.[19]

The unique spectroscopic profile of **6a** allowed us to identify with a high degree of certainty the coupled material during HPLC studies.[16,19] However, isolation and characterization of Staudinger adducts derived from both **6a** and **6b** revealed that neither material gave rise to the anticipated adducts **8a** or **8b** following reaction with **7**. Careful elucidation of purported **8b**, in the way of high-resolution NMR experiments (gDQF-COSY, ROESY, gHMBC, and gHSQC) afforded data consistent with *O*-methyl imidate **9b** as the Staudinger adduct resulting from reaction of **6b** and **7**. *Significantly, the alkoxide moiety (Figure 2, red highlight) typically lost during Staudinger coupling of alkyl azides is retained in the coupling of C8 azidoadenosine.*

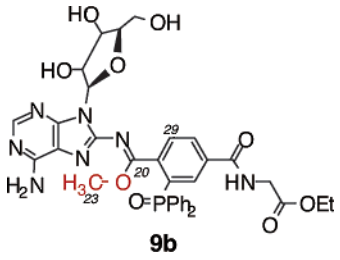


Figure 2. *O*-Methyl imidate Staudinger ligation adduct.

The expediency of this coupling and the stability of the adduct formed promoted our further interest in terms of scope and limitations. Of particular significance is that Staudinger ligations involving aryl azides do not require the use of the disubstituted phosphine developed by Bertozzi and coworkers. Simple esterification of commercially available triarylphosphines with “capped” linkers of interest would allow a species still capable of coupling two abiotic substances, but whose ligation is more facile than the corresponding alkyl azide case and for which phosphine

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(17) Others in the field have also exploited wet THF to enhance the solubility of coupling components.[9]
(18) Triarylphosphines **6a** and **6b** were prepared in accordance with refs 9 (phosphine construction) and 10 (phosphine linkage) with only subtle modification to amide construction.
(19) See Supporting Information.

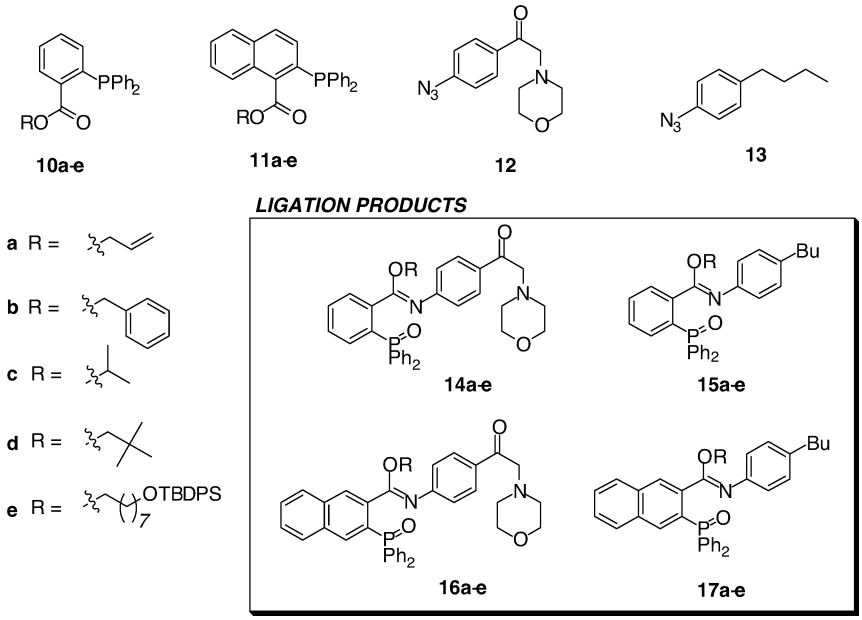


Figure 3. Aryl azides, triarylphosphines, and corresponding ligation adducts isolated after chromatography.

construction is as simple as esterification. Widespread application of this precise coupling dictates, however, that azide and phosphine substitution patterns be examined with respect to coupling efficiency and imidate stability under aqueous conditions.

2-(Diphenylphosphino)benzoic acid and 2-diphenylphosphino-1-naphthoic acid are both commercially available and readily esterified via subjection to a mixture of PPh_3, DIAD, and a suitable alcohol.[19] As such, we constructed the panel of phosphino esters **10a**−**e** and **11a**−**e** and evaluated the amenability of these substances to undergo Staudinger coupling/ligation with aryl azides **12** and **13** (Figure 3). It is important to note that 4-azidophenacyl morpholine is derived from the classical photoaffinity label 4-azidophenacyl bromide and that aryl azides in general have enjoyed widespread and long-term popularity as photoactive labeling agents for biomolecules. Table 1 reveals the interesting result that phosphino naphthoic esters afford more stable imidate adducts than do the related triphenylphosphino esters. Although analysis of crude reaction products by TLC and ^{1}H NMR revealed that all Staudinger couplings proceed with comparable efficiencies, the imidates resulting from condensation of butyl azide **13** and triphenylphosphino esters **10a**−**e** were not amenable to chromatographic purification. Following chromatography, low yields (<20%) of unstable imidates were obtained contributing to already complicated mixtures of products. Only in the ligations of **10a** and **10b** with **13** could the initially anticipated amide product (**15f**)[19] be identified in significant quantities (37% yield for **10a** and 67% yield for **10b**). In the majority of cases examined, imidates are isolated in yields moderate by organic synthesis standards but certainly high enough to support the importance of this coupling in biologically oriented studies.

Why are imidates the principal products of Staudinger ligation with aryl azides? In formulating a cogent answer to this, we considered the previously hypothesized mechanism for reaction of alkyl azides with phosphines bearing the core structure **1**, as well as mechanisms recently proposed for Staudinger reduction/intramolecular aza-Wittig (S-AW) processes used in the successful total syntheses of Apratoxin and (−)-Ephedradine A.[8,20,21] The substitution patterns of **6a**,**b**, **10a**−**e** and **11a**−**e** coupled with the aromatic nature of azides examined here provide an alternative mechanistic foundation for our findings.

Reaction of tertiary phosphines with azides ordinarily produces an iminophosphorane such as **3** (Scheme 1)

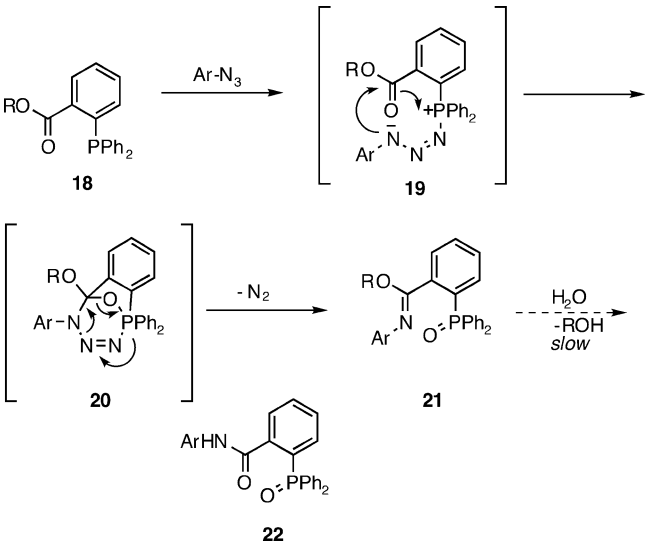
Table 1. *O*-Alkyl Imidate Yields from Staudinger Ligation of Aryl Azides

phosphine	azide	product	yield
10a	12	**14a**	64%
10b	12	**14b**	53%
10c	12	**14c**	66%
10d	12	**14d**	67%
10e	12	**14e**	66%
11a	12	**16a**	64%
11b	12	**16b**	62%
11c	12	**16c**	40%
11d	12	**16d**	38%
11e	12	**16e**	55%
10a	13	**15a**	
10b	13	**15b**	
10c	13	**15c**	
10d	13	**15d**	
10e	13	**15e**	
11a	13	**17a**	65%
11b	13	**17b**	60%
11c	13	**17c**	55%
11d	13	**17d**	40%
11e	13	**17e**	58%

following N_2 evolution.[22] The primary imination products, retaining the azide-derived nitrogen triad, are sometimes sufficiently stable to permit isolation. As a rule though, these phosphazides proceed rapidly to the iminophosphorane via loss of N_2. Significantly, phosphazides derived from aryl azides and triarylphosphines have been effectively trapped through formation of transition metal complexes (Pd, V, W, Zr) and are well-known to undergo intramolecular electrocyclic reactions affording access to extended heterocyclic systems.[22−25] Isolable phosphazides have been formed from sterically hindered azides and phosphines or in cases where phosphorus electron density has been increased.[23] Substituent-dependent decreases in azide Nα electron density also correlate to phosphazide stability.[23] Steric bulk, in particular, is believed to play an important role in abrogating formation of the betaine necessary for N_2 liberation.

Given this, we propose initial generation of the extended azaphosphonium ylide **19**, the viability of which is supported by seminal mechanistic work by Temple and Leffler.[26] Model building reveals that the arylamine of **19** is in close proximity to the carbonyl of **19** via a seven-membered transition state with the azide-derived triad in the thermodynamically more stable cis conformation.[27] The *o*-carboalkoxy moiety serves then to quench the negatively charged Nα center, and the resulting tetrahedral intermediate immediately satisfies charge on the positively charged phosphorus, thus rendering bicyclic **20**. Alternatively, this process can be viewed as an electrocyclic closure to **20**. The phosphazido oxide **20** is proposed to decompose in short order via loss of nitrogen and concomitant installation of the phosphine oxide moiety in a fashion closely paralleling Molina's synthesis of carbodiimides from α-azidodiphenylacetonitrile and triphenylphosphine.[28] The resultant **21** is very often, in our hands, a stable material highly resistant to hydrolysis to **22**. That N_2 evolution is the final step in this path contrasts sharply with the mechanism shown in Scheme 1 (and most S-AW processes), wherein loss of N_2 precedes formation of bicyclic **4** (Scheme 1). Such an intermediate, or a closely related one, is supported by ^{31}P NMR data,[8] which was unattainable in our experiments due to mechanistic independence from a hydrolytic step.[29] Thus, mechanistic and functional differences of Staudinger ligations with alkyl versus aryl azides appear to be quite profound. At the heart of this difference is the significantly enhanced stability, attenuated nucleophilicity, and greater steric bulk of aryl phosphazides relative to their alkyl analogues.

Scheme 2



In summary, we demonstrate here the application of a Staudinger reaction in obtaining ligated materials from aryl azides. More importantly, the principal product that results from these ligations bears a relatively stable imidate linkage. The preference of aryl azides to give rise to imidate over amide Staudinger ligation products is likely a reflection of steric and electronic differences between the two types of azides. Modulation of these differences is expected to impact product profiles and must clearly be considered in the design of new abiotic coupling reagents. Studies detailed herein suggest the importance of simple esterification of commercially available triarylphosphines en route to a simplified and more broadly accessible Staudinger ligation scenario for use in chemical biology.

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Supporting Information Available: Experimental procedures and accompanying characterization for all new materials and representative HPLC traces for reactions involving phosphine **6a**, **7**, and related substances highlighted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) As initially ascertained by HPLC-MS experiments, which showed that use of $^{18}OH_2$ during ligation/coupling of **6a** to **7** did not lead to detectable ^{18}O incorporation into **9a**. This result was independently supported by ^{31}P NMR experiments in which trace amounts of H_2O (as a component of THF-d_8) did not get consumed during the coupling of **6b** and **7**.