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Triphenylphosphinecarboxamide: An Effective Reagent for the Reduction of Azides and Its Application to Nucleic Acid Detection

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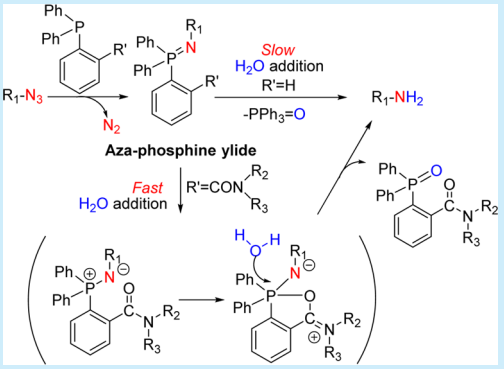
Supporting Information

ABSTRACT: A series of triphenylphosphinecarboxamide (TPPc) derivatives were designed and synthesized as alternative reagents to triphenylphosphine for the facile reduction of azides. The TPPc derivatives performed as efficient reducing agents for the synthesis of primary amines without the need for an additional hydrolysis procedure. The TPPc derivatives were also applied to nucleic acid sensing using a RhAz-oligonucleotide conjugate in a DNA-templated fluorogenic reaction.



The reduction of an azide group to the corresponding amine represents an indispensable reaction in organic synthesis, and the Staudinger reaction, especially, continues to be an important transformation in both modern organic synthesis and bioorganic chemistry.[1,2] There are several attractive features to the Staudinger reaction, including the fact that the reaction requires only very mild conditions that are compatible with other hydrogenolytically sensitive functional groups such as benzyl type protecting groups and double bond moieties. In addition, the bioorthogonal properties of the Staudinger reaction have been employed in chemical ligations (i.e., Staudinger-Bertozzi ligation)[3−5] and molecular sensing, where azide masked fluorogenic compounds were used in oligonucleotide templated reactions.[6−15] The iminophosphorane intermediate formed during the Staudinger reaction, however, can be particularly stable toward hydrolysis with H_2O and can require relatively long reaction times to be completely converted to the corresponding primary amines. For convenience, acidic or basic conditions are generally used to hydrolyze the iminophosphoranes.[16] During the synthesis of multifunctional target molecules or analytical systems such as those used in DNA detection, for example, the requirement for additional treatments can be time-consuming, and the treatments themselves could have an adverse impact on some of the other functional groups present in the system. Furthermore, it is not possible to use post-treatment processes of this type in a cellular context. One of the alternatives to using triphenylphosphine is to use trialkyl phosphines such as triscarboxymethylphosphine (TCEP), which has been widely used as a reducing reagent in biochemistry.[17] Unfortunately, alkyl phosphines are unstable and can be readily oxidized in air as well as aqueous solution.[11]

With this particular limitation in mind, our attention became focused on the impact of introducing substituents at the *ortho* position of the phenyl rings of triphenylphosphine (TPP) to assist in the hydrolysis of the intermediate iminophosphorane through neighboring group participation (NGP). One of the most successful examples of NGP in this context was a Staudinger-Bertozzi ligation, where an *o*-methylcarboxydiphenyl phosphine derivative was used to facilitate an intramolecular migration to form a covalent amide bond.[3−5] Interestingly, the results of this particular study indicated that the addition of water was also required to facilitate this reaction. This study inspired us to want to develop a new triphenylphosphine-based derivative for the reduction of azides. As shown in Figure 1, it was envisaged that the use of a carboxamide instead of an ester

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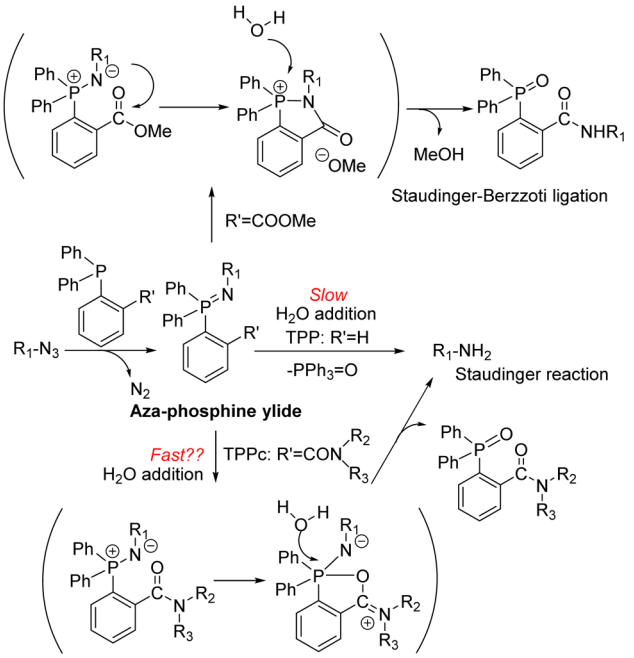
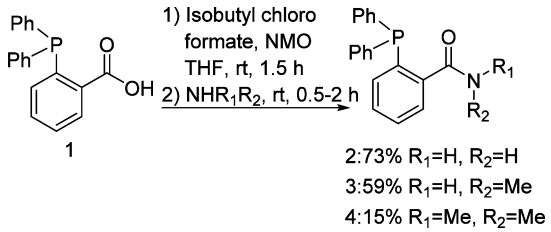


Figure 1. Azide reduction via neighboring group participation.

as the *ortho* substituent would prevent the migration of this substituent because of its poor migratory aptitude of the carboxamide group. Furthermore, the carboxamide moiety has been shown to assist in NGP[18] and could promote the rapid hydrolysis of the iminophosphorane intermediate without the requirement for any additional treatment processes. In this study, a series of triphenylphosphine carboxamide (TPPc) derivatives were designed and synthesized to investigate the feasibility of our new mechanism. These TPPc derivatives were subsequently used to reduce azides without the requirement for an additional hydrolysis treatment in nucleic acid sensing.

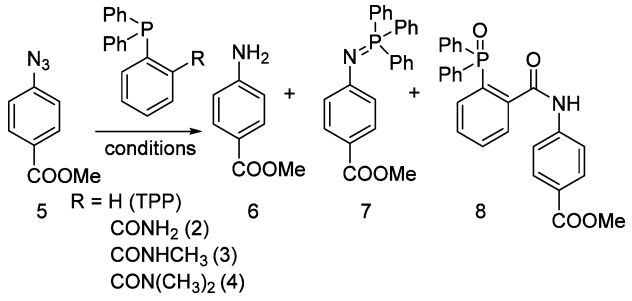
For the synthesis of the TPPc derivatives, *o*-diphenyl phosphino benzoic acid **1** was subjected to a series of coupling reactions with ammonia, methyl amine, and dimethyl amine using a mixed anhydride intermediate to give the desired TPPc derivatives **2**–**4**, as shown in Scheme 1.

Scheme 1. Synthesis of Triphenylphosphine Carboxamide Derivatives



At first, we compared the reduction ability of TPP with those of the TPPc derivatives (**2**–**4**) under conditions A (anhydrous THF and then H_2O) and B (THF/H_2O, 9:1, v/v) (Table 1). TPP reacted with methyl azide benzoate **5**[19] to give aza-ylide complex **7** in quantitative yield under both sets of conditions. Surprisingly, the aza-ylide complex **7** remained intact even in the presence of water (Table 1, entry 2). Furthermore, when the reaction time was extended to 24 h, the aza-ylide complex was obtained in 95% yield (Table 1, entry 3). On the other

Table 1. Model Staudinger Reaction using TPP and the TPPc Derivatives[a]



entry	R	solvent	time (h)	**6** (%)	**7** (%)	**8** (%)
1	H	A	2		98	
2	H	B	2		94	
3	H	B	24		95	
4	$CONH_2$	A	2	65		28
5	$CONH_2$	B	2	91		
6[b]	$CONH_2$	B	10 min	93		
7	$CONHCH_3$	A	2	51		21
8	$CONHCH_3$	B	2	58		
9	$CON(CH_3)_2$	A	2	38		5
10	$CON(CH_3)_2$	B	2	45		

[a]Conditions: **5** (0.1 M), TPP or TPPc **2**–**4** (1.1 equiv); solvent: A {THF (5 mL) and quenched with H_2O} or B {THF/H_2O (5 mL), 9:1, v/v}. [b]1.5 equiv of **2** was used.

hand, TPPc **2** gave the reduced compound **6** (65%) as well as the ligated product **8** (28%) under condition A. When aqueous conditions were used, the reduced compound **6** was obtained in excellent yield (91%) without any of the aza-ylide complex or ligated product being detected. Furthermore, when a slight excess of reducing reagent **2** (1.5 equiv) was used, the reaction reached completion within 10 min to give compound **6** in 93% yield (Table 1, entry 5). Pseudo-first-order condition was applied and checked by ^{31}P NMR. The result revealed that the phosphine was rapidly converted to the phosphine oxide (5 min) with the aza-ylide complex not being detected (Supplementary Figure S1). The difference between these results was attributed to the amide group, which presumably assisted in the nucleophilic attack of water on the aza-ylide complex. To clarified the effect of the amide structure, we also tested the secondary and tertiary amides **3** and **4**. Pleasingly, similar tendencies were observed for these amides in terms of the formation of the aza-ylide complex (Table 1, entries 7–10). A comparison of the reactivity of these TPPc derivatives revealed that their reactivity was dependent on the number of substituents on the nitrogen atom. Thus, as the number of substituents on the nitrogen increased, there was a reduction in the yield and reactivity of the TPPc derivative toward the azide group.To assess the overall scope and applicability of this new method, several substrates were tested, including a functional alkyl azide, fluorescent molecule, aromatic azide, carbohydrate, and nucleoside. Pleasingly, the results revealed that the reduction conditions described above were tolerant of a variety of different functional groups, including benzyl, silyl, imide, dimethoxytrityl, double bond, acetal, and carbamate moieties (Table 2). Furthermore, the current reduction system eliminated the requirement for an additional treatment process to decompose the aza-ylide complex typically formed when TPP is used as the reducing agent. Taken together, these results demonstrate that the TPPc derivative **2** could be useful for the

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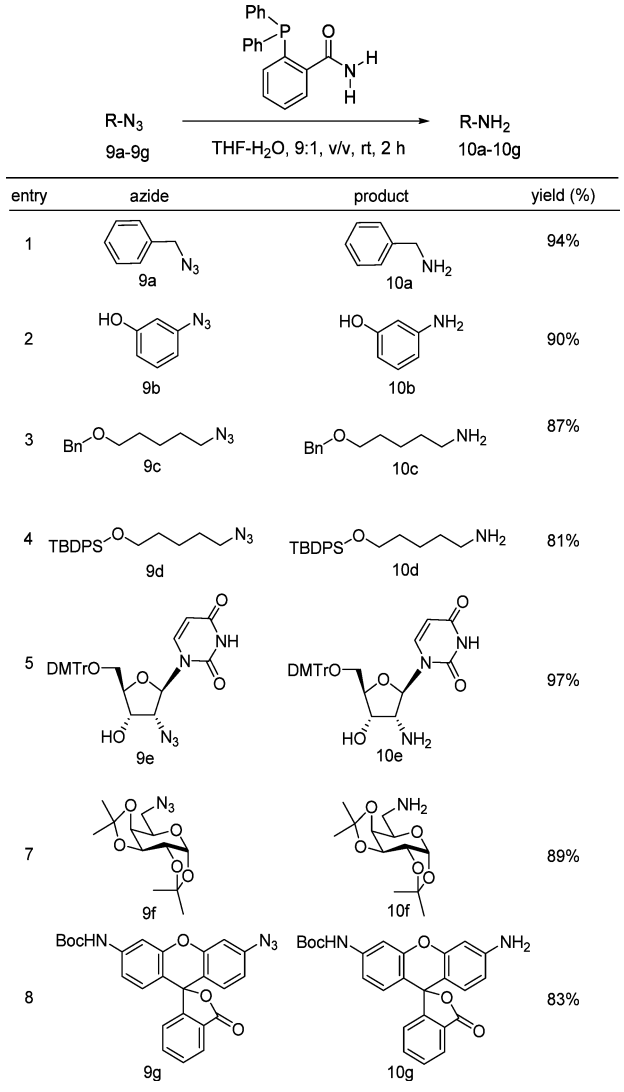
Table 2. Azide Reduction of Different Azide-Containing Compounds[a]



[a]Conditions: **9a–9g** (0.1 M), phosphine **2** (1.1 equiv), THF/H_2O (9:1, v/v), rt, 2 h.

reduction of azide groups during the synthesis of multifunctional molecules. We then proceeded to investigate the use of TPPc for the detection of nucleic acid species using a rhodamine azide (RhAz)-oligonucleotide probe in a biological buffer. Several other groups including our own have already reported the formation of an aza-ylide complex during a DNA templated reaction using a TPP-oligonucleotide probe and its fluorescence peak, which appears around 550 nm during fluorescence analysis instead of 525 nm, which was previously reported for rhodamine110.[8,15] If the TPPc derivative worked as suggested in Scheme 1, then none of the aza-ylide complex would be formed. In addition, the fluorescence intensity of the aza-ylide complex would be lower than that of the fully reduced species.[8]

The new RhAz-TPPc probe pair gave a fluorescence signal that was very similar to that of dithiothreitol, indicating that none of the aza-ylide complex had been formed (Figure 2). In contrast, the RhAz-TPP pair gave a smaller fluorescence signal because of the formation of the aza-ylide complex.

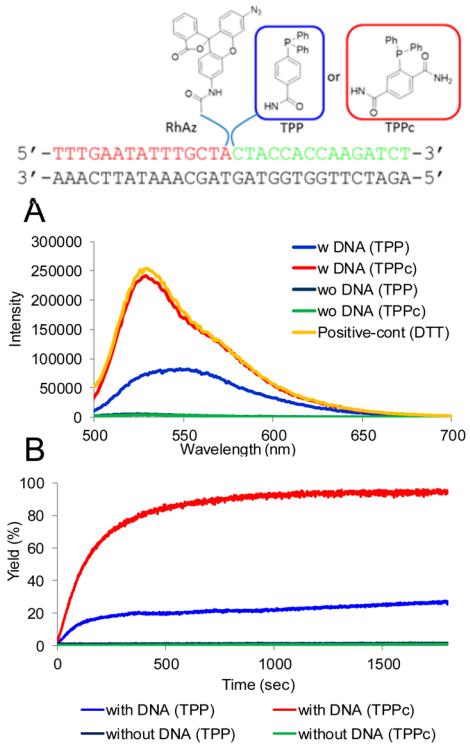


Figure 2. DNA-templated Staudinger reaction by conventional RhAz-TPP or new RhAz-TPPc. (A) Fluorescence spectrum of reaction mixture after 30 min. (B) Time course of DNA-templated Staudinger reaction. Conditions: 100 nM RhAz probe, 300 nM TPP or TPPc probe, 100 nM DNA target in 20 mM Tris-HCl (pH 7.2) containing 100 mM $MgCl_2$ and 10 μg/mL BSA, 37 °C, 30 min, ex 490 nm, em 525 nm. Yield was calculated from reduced RhAz probe by dithiothreitol (DTT).

The reaction yield of the RhAz-TPPc pair was determined to be almost quantitative after 30 min. The greatest advantage of the RhAz-TPPc pair over the RhAz-TPP pair was its signal amplification ability, as shown in Supplementary Figures S2 and S3. Even when the target DNA was present as 0.1 equiv, the same reaction yield was similar to that achieved under stoichiometric conditions. In contrast, the conventional RhAz-TPP pair provided a much lower level of signal amplification. The proposed mechanism for this transformation is shown in Supplementary Figure S4. The TPP-RhAz pair (conventional) would generate an aza-ylide complex, which would disturb the strand exchange process and result in reduced levels of signal amplification. These results indicated that the RhAz-TPPc pair provided an enhanced level of signal amplification based on fast aza-ylide hydrolysis, making it much more suitable for use in nucleic acid detection compared with the conventional TPP-RhAz probe pair.

In summary, we have developed a series of new TPPc derivatives that can be used to reduce azides to the corresponding amines under very mild conditions in a single step. In contrast to the conditions traditionally used for the reduction of azides by TPP, where an additional hydrolysis reaction is required to break down any intermediate aza-ylide complex, our new strategy successfully avoids this additional step and allows for direct access to primary amines in complex functional molecules. Further applications, such as those involving the reduction of the azide group in the protein, are currently being conducted in our laboratory.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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