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A new drug-release method using the Staudinger ligation

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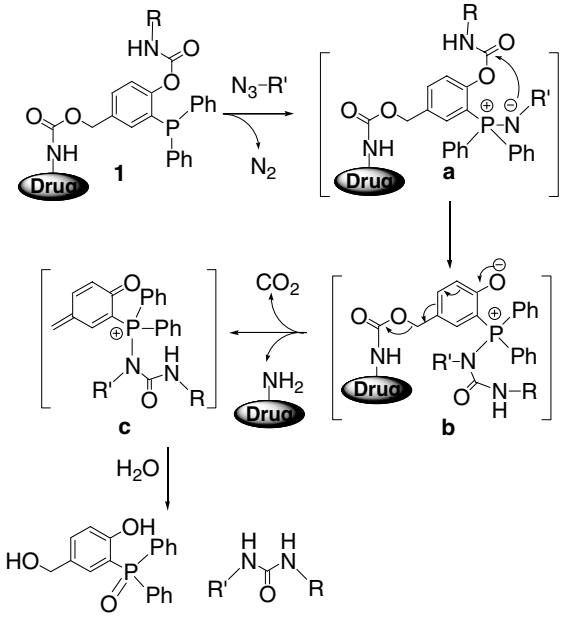
Abstract—Many drugs induce severe side-effects caused by their lack of selectivity. One way to overcome this problem is to design a specific system which releases a free drug in a controlled manner. Herein we describe a new way to liberate a drug from a prodrug using the Staudinger ligation as the trigger.

The Staudinger reaction was developed in 1919 by Staudinger and Meyer.[1,2] This reaction consists in the nucleophilic attack of a phosphine on an azide leading to a phosphazide which, through the loss of nitrogen, gives a reactive aza-ylide. In the presence of water, this intermediate spontaneously hydrolyzes to yield a primary amine and the corresponding phosphine oxide. Saxon and Bertozzi reported a modification of the Staudinger reaction for the first time in 2000, 'the intramolecular Staudinger ligation'.[3,4] The elegant modification consists in positioning an electrophilic trap, such as a methyl ester, into the phosphine structure in order to capture the aza-ylide intermediate by an intramolecular cyclization. This reaction is compatible with a large number of functional groups and therefore has various uses in organic synthesis and biological chemistry. The Staudinger ligation was applied to peptide synthesis[5,6] or to the preparation of carbohydrate–protein conjugates.[7] The modified Staudinger reaction has already been used for the immobilization of substrates to surfaces,[8,9] in the investigation of cellular metabolism of synthetic azido-sugars,[10] also for biological labeling[11,12] and even on living organisms such as mice.[13]

Here, we propose a new application of the Staudinger ligation to trigger drug liberation from a prodrug. The main goal of this project is to release a free drug in a stringently controlled manner. For this purpose, the Staudinger ligation was carried out with a modified electrophilic trap, as a phenyl carbamate to capture the aza-ylide intermediary formed. We expected the intramolecular cyclization to generate an hydroxy–benzylic–carbonyl system, known to initiate a 1,6-elimination process.[14,15] This kind of self-eliminating linker[16–18] is widely used in drug targeting.

We designed a phosphine **1** as a potential prodrug that would enable a new rearrangement of the aza-ylide **a** (Scheme 1). We postulated that this aza-ylide **a** would generate the phenol anion **b** which would undergo a



Scheme 1. Postulated mechanism of prodrug activation via the Staudinger ligation.

Keywords: Prodrug; Staudinger ligation; Doxorubicin; Drug releaser.
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1,6-quinone methide rearrangement, followed by spontaneous decarboxylation to liberate the drug entity. Then the quinone methide **c** would be hydrolyzed by water to form urea and phosphine oxide compounds.

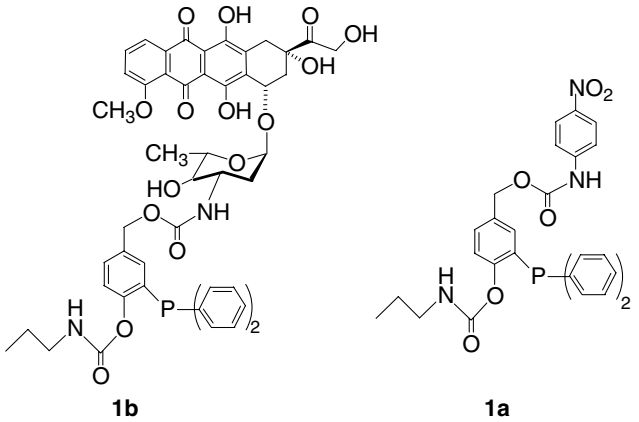
To provide a proof of this concept, we synthesized the two following compounds (Scheme 2): **1a**, harboring a 4-nitroaniline (pNA) as a model compound representing a potential amine-drug; **1b**, carrying doxorubicin as a drug. We then tested a model reaction between these two phosphine compounds **1a** or **1b** and the commercially available azido-compound **2** in aqueous THF (1:1) at 37 °C.

The synthesis of the prodrug analogue **1a** is described in Scheme 3. The commercially available compound **3** was reacted with iodine monochloride[19] to yield the monoiodoaldehyde **4**. The tertiary phosphine **5** was obtained by palladium-catalyzed cross-coupling between diphenylphosphine and the iodo-compound **4** as described by Herd et al.[20] Condensation of compound **5** with the commercially available *n*-propylisocyanate in the presence of a catalytic amount of pyridine[21] led to the carbamate **6**. Reduction of compound **6** by $NaBH_4$ in a mixture of chloroform and 2-propanol[22] produced the corresponding alcohol **7**. The latter was mixed with the 4-nitrophenylisocyanate to generate the 4-nitroanilinecarbamate as the model compound **1a**.

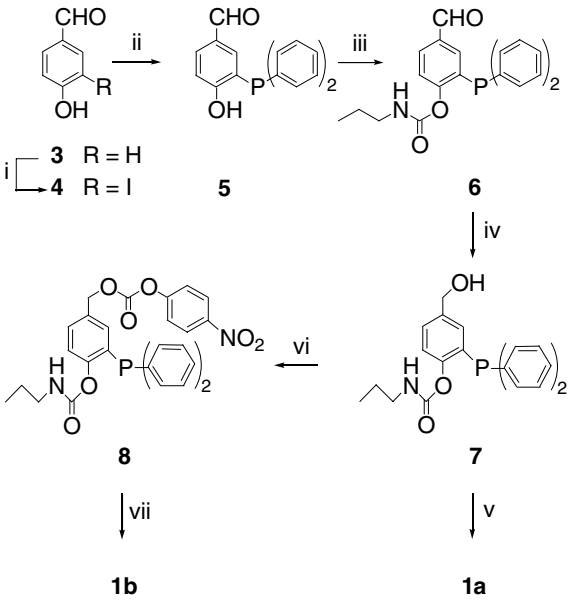
The doxorubicin prodrug **1b** was obtained in two steps from compound **7**, as shown in Scheme 3. Acylation of the alcohol **7** with 2 equivalents of 4-nitrophenylchloroformate[23] produced the carbonate **8**, which was then reacted with doxorubicin to give the expected prodrug **1b**.

With these two compounds in hand, we first examined the reaction between the model compound **1a** and the water-soluble azido-compound **2** (Fig. 1).

When **1a** was incubated with compound **2**, the increase in absorbance was immediately obvious. 4-Nitroaniline formation was monitored by UV spectroscopy at 405 nm wavelength.[24] The released 4-nitroaniline clearly showed that compound **2** initiated an intracyclization followed by a 1,6-quinone methide rearrangement,



Scheme 2. Structures of the potential amine-prodrug **1a** and the doxorubin prodrug **1b**.



Scheme 3. Reagents: (i) ICl, CH_2Cl_2, 60%; (ii) Ph_2PH, CH_3CN, $Pd(OAc)_2$, KOAc, 44%; (iii) *n*-propylisocyanate, CH_2Cl_2, 91%; (iv) $NaBH_4$, $CHCl_3/CH_3(CH_2)_2OH$ (5:1), 85%; (v) 4-nitrophenylisocyanate, CH_2Cl_2, 60%; (vi) 4-nitrophenylchloroformate, pyridine, CH_2Cl_2, 58%; (vii) doxorubicin, DMF, Et_3N, 33%.

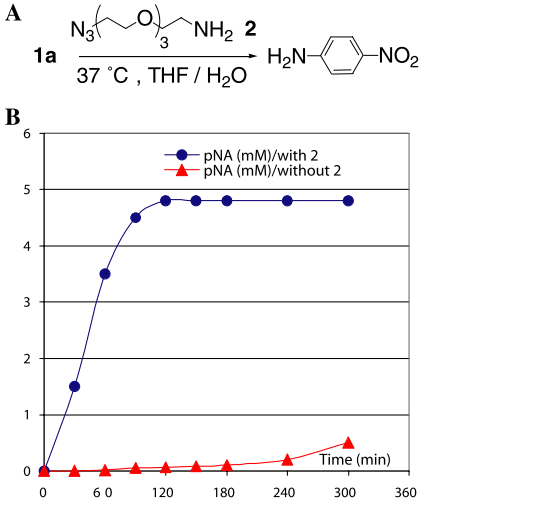


Figure 1. (A) Incubation of compounds **1a** (5 mM) and **2** (10 mM); (B) pNA release was monitored by its absorbance, as a function of time, with or without **2**.

according to the proposed pathway presented in Scheme 1. Without **2**, no absorbance of pNA was observed.

In the second experiment, we tested the same reaction with the doxorubicin prodrug **1b** (Fig. 2).

The release of the free doxorubicin was monitored by reverse-phase HPLC. As in the first experiment, no drug liberation was observed in the absence of the azido-compound. Doxorubicin liberation was the second evidence that the reaction followed the mechanism illustrated in Scheme 1. To evaluate the efficiency of the activation, we quantified the drug release as described in

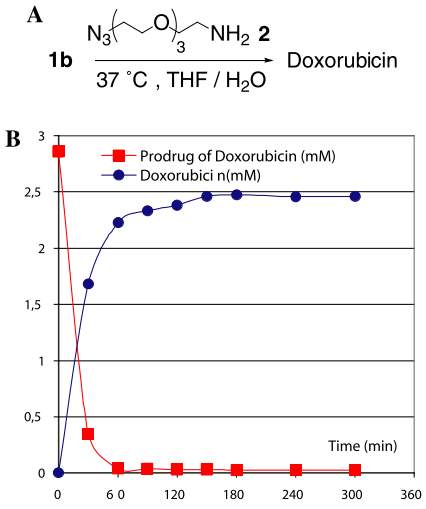


Figure 2. (A) Incubation of compounds **1b** (2.8 mM) and **2** (5.2 mM). (B) Doxorubicin release was monitored by HPLC analysis, as a function of time.

Supplementary data. After 3 h at 37 °C in aqueous THF, the reaction appeared to approach 90% completion.

In summary, we have successfully demonstrated for the first time, the utility of the Staudinger ligation to liberate a drug selectively and in good yield. In this report, we have used an experimental model. We now wish to extend our results to the complex environment of a living cell. The Staudinger ligation seems perfectly suitable for such an application, since the two reaction partners are biocompatible and orthogonal to most biological functionalities. Further studies to take advantage of this new drug-release method are currently in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.073.

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