The azaquinone-methide elimination: comparison study of 1,6- and 1,4-eliminations under physiological conditions†

Rotem Erez and Doron Shabat*

Received 14th May 2008, Accepted 12th June 2008
First published as an Advance Article on the web 23rd June 2008
DOI: 10.1039/b808198k

The azaquinone-methide elimination is a powerful and efficient reaction useful for disassembly of spacers in prodrug systems. We and others have used the spacer-technique to develop dendritic and polymeric self-immolative molecular systems that can disassemble through a domino-like mechanism upon a stimulus event. In this report, we study the disassembly of a system that can disintegrate through *para*- and *ortho*-azaquinone-methide eliminations. The disassembly was evaluated with molecules that undergo single 1,6- or 1,4-elimination and with molecules that undergo double 1,6- and 1,4-elimination. The 1,6-elimination was slightly faster than the 1,4-elimination under physiological conditions. This study sheds light on the disassembly-behavior of prodrug systems.

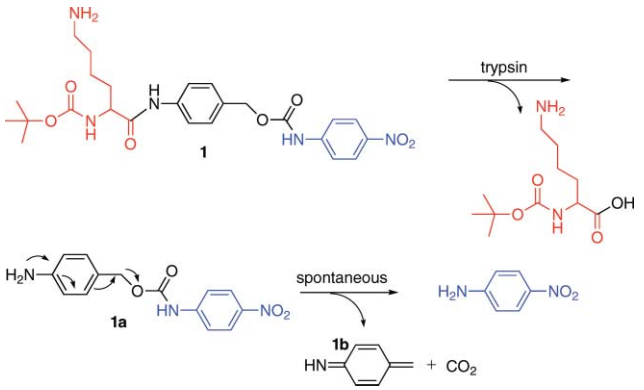

Fig. 1 Activation mechanism of a tripartate prodrug by enzymatic cleavage and elimination through an azaquinone-methide species.

In 1981, the group of Katzenellenbogen discovered that 4-amino-benzyl alcohol can function as an efficient self-immolative spacer in prodrug design. The spacer is used to link a drug to an enzymatic substrate, thereby generating a tripartate prodrug.[1] The prodrug is stable as long as the enzymatic substrate is attached. However, cleavage of the substrate by the enzyme generates an intermediate that rapidly releases the free active drug. The concept was demonstrated with 4-nitroaniline as a model drug and lysine as an enzymatic substrate for trypsin (Fig. 1). Incubation of compound **1** with trypsin resulted in release of amine **1a** that spontaneously underwent 1,6-elimination to generate azaquinone-methide **1b** and free 4-nitroaniline.

Since this discovery, numerous scientists have used this spacer-technique for prodrug design.[2] It was shown that 4-hydroxy-benzyl alcohol and 4-mercapto-benzyl alcohol also undergo 1,6-elimination upon exposure of the hydroxyl or the thiol groups.[3,4] When 2-amino-benzyl alcohol is used as a spacer, the system can undergo analogous 1,4-elimination through an *ortho*-azaquinone-methide. We have used the spacer technique to develop dendritic and polymeric self-immolative molecular systems that disassemble through a domino-like mechanism upon a stimulus event.[5–7] The systems act as molecular amplifiers for diagnostic and drug delivery applications.[8,9] The azaquinone-methide elimination was shown to serve as a powerful and efficient reaction in the design of self-immolative molecular systems.[10] The design of a self-immolative dendritic adaptor was based on a molecule where both 1,4 and 1,6-elimination reactions occurred in the same aromatic ring (Fig. 2). In this contribution, we report a comparison study of the reaction rates of *para*- and *ortho*-azaquinone-methide eliminations. Compound type **I** (Fig. 2) has two reporter groups (R) on the benzylic substituents, located at the *para* and *ortho* positions of the corresponded aniline. An amide linkage with an enzymatic substrate blocks the aromatic-amino group. Removal of the substrate by the enzyme initiates the disassembly of the system through *para* and *ortho*-azaquinone-methide eliminations to release the two reporters.

In order to compare the rates of 1,4 and 1,6-elimination, we initially synthesized two simple model compounds **2** and **3** (Fig. 3, see Supporting Information for synthesis details†). Both compounds have a trigger group that can be removed through cleavage of the phenylacetamide catalyzed by penicillin-G-amidase (PGA).[11] Enzymatic cleavage is followed by spontaneous 1,4- or 1,6-elimination and decarboxylation to release the corresponded aniline (the 4-amino-benzyl alcohol unit is used as a spacer between the aniline and the phenylacetamide enzymatic substrate). While aniline **2a** undergoes 1,6-elimination to release the 5-amino-2-nitrobenzoic acid, aniline **3a** disintegrates through 1,4-elimination.

Compounds **2** and **3** were incubated in phosphate buffer saline (pH 7.4; PBS) with PGA and the release of 5-amino-2-nitrobenzoic acid was monitored by RP-HPLC (Fig. 4). Compound **2** disassembled within 30 min without any detectable intermediate to release the reporter unit (Fig. 4A). The disassembly of compound **3** under the identical conditions occurred over 60 min and the appearance and disappearance of intermediate **3a** were observable (Fig. 4B). These results show that the reaction-rate of the 1,4-elimination is slightly slower than that of the 1,6-elimination (the identification of

Department of Organic Chemistry, School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel Aviv 69978, Israel. E-mail: chdoron@post.tau.ac.il; Fax: +972 (0) 3 640 9293; Tel: +972 (0) 3 640 8340

† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/b808198k

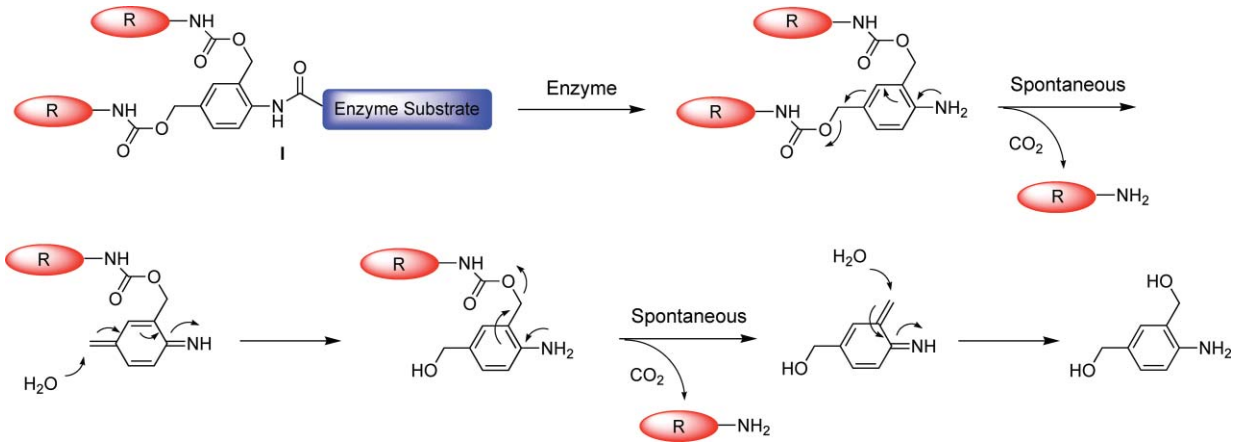

Fig. 2 Disassembly of compound type **I** through 1,6- and 1,4-elimination reactions to release the reporter units.

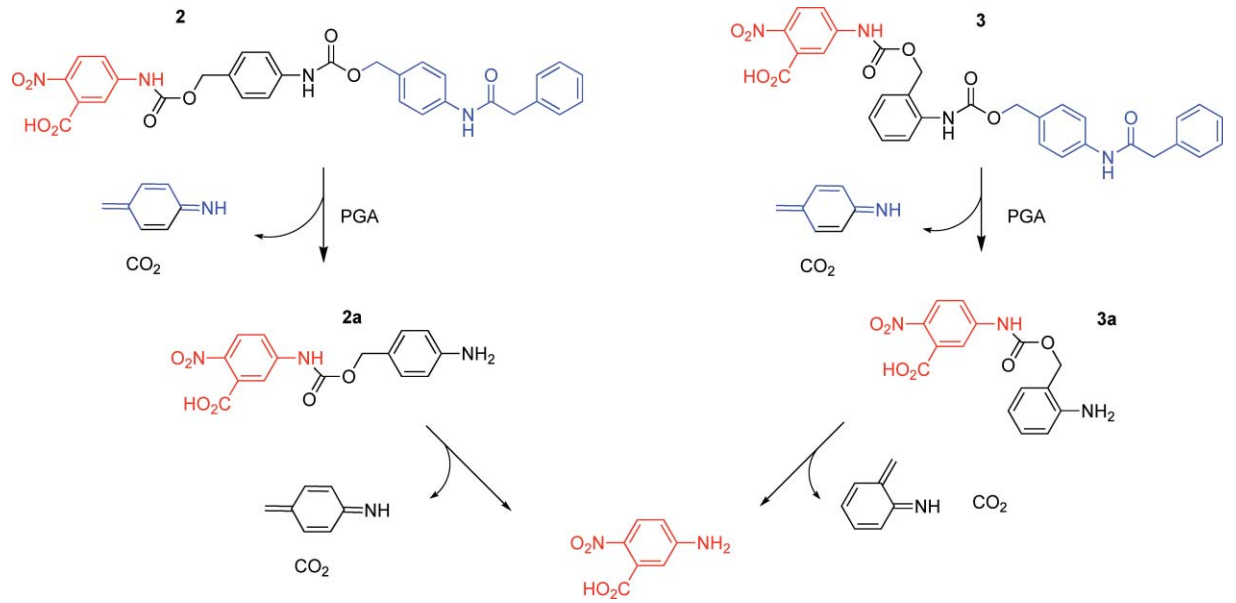

Fig. 3 PGA-catalyzed disassembly of compound **2** through 1,6-elimination and of compound **3** through 1,4-elimination to release 5-amino-2-nitrobenzoic acid.

intermediate **3a** was confirmed through its UV spectrum observed by the photo-diode array detector of the HPLC).

With these results in hand, we evaluated the disassembly of compound type **1**. Compounds **4** and **5** were synthesized (see Supporting Information†) with a triggering group designed for cleavage by PGA (Fig. 5). Upon activation, each of the compounds can undergo 1,4 and 1,6-elimination reactions to release its reporter units according to Fig. 1. Two different reporters were introduced on each one of compounds **4** and **5**. On compound **4**, the reporter 5-amino-2-nitrobenzoic acid was linked at the *para*-benzyl substituent and the reporter 4-nitroaniline at the *ortho* one. On compound **5**, 4-nitroaniline was linked at the *para*-benzyl substituent and 5-amino-2-nitrobenzoic acid at the *ortho* one.

Compounds **4** and **5** were incubated in PBS (pH 7.4) with PGA and the release of 5-amino-2-nitrobenzoic acid and 4-nitroaniline was monitored by RP-HPLC (Fig. 6). As shown in Fig. 6A, most of compound **4** disappeared within 1 h. The reporter 5-amino-2-nitrobenzoic acid, linked at the *para*-benzyl substituent, was released faster than the *ortho*-linked 4-nitroaniline. For compound **5**, the *para*-linked 4-nitroaniline unit was released more rapidly than the 5-amino-2-nitrobenzoic acid (Fig. 6B). In both examples, the disassembly was complete after approximately six hours. The concentration of PGA used, 0.01 mg mL^{-1}, allowed the enzymatic cleavage on a time-scale suitable for monitoring of the reaction by HPLC.

The results obtained in this study clearly indicate that the 1,6-elimination through the *para*-azaquinone-methide occurs faster than the 1,4-elimination through the *ortho*-azaquinone-methide species. Although compound **2** disassembled rapidly through 1,6-elimination with no detectable intermediate, the disassembly of compound **3** through 1,4-elimination occurred more slowly and the generation of intermediate **3a** was observed. The disassembly

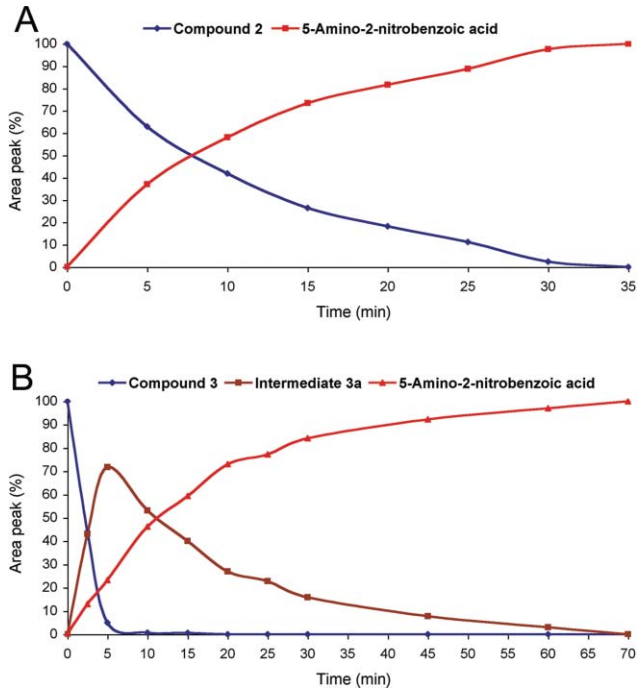

Fig. 4 PGA-catalyzed the release of 5-amino-2-nitrobenzoic acid from compounds **2** and **3** through 1,6- and 1,4-eliminations respectively. Conditions: **2** or **3**, [500 μM] in PBS 7.4 and PGA [0.01 mg mL^{-1}].

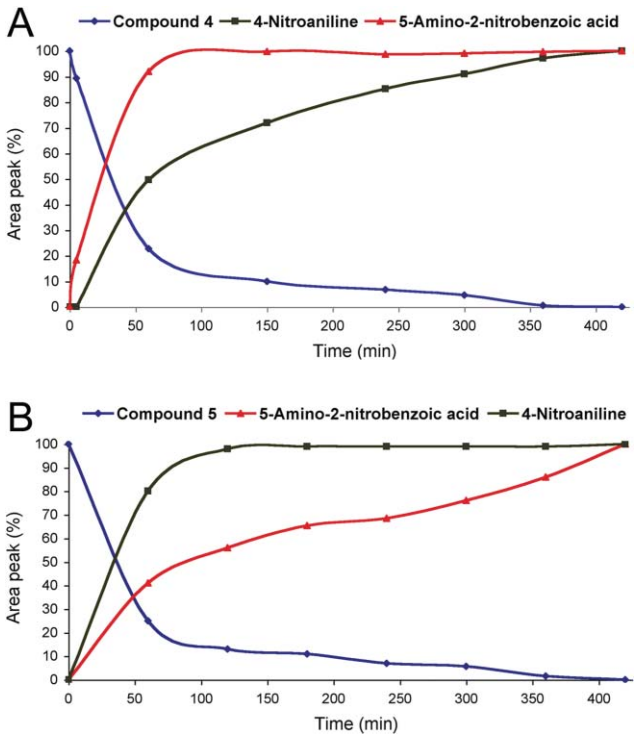

Fig. 6 PGA-catalyzed the release of 5-amino-2-nitrobenzoic acid and 4-nitroaniline from compounds **4** and **5** through 1,6- and 1,4-eliminations. Conditions: **4** or **5**, [500 μM] in PBS 7.4, PGA [0.01 mg mL^{-1}].

of compounds **4** and **5**, which bear two different substituents to allow independent analysis of 1,4- and 1,6-elimination reactions of the same compound, occurred with a faster release of the *para*-reporter than the *ortho* one, indicating that the 1,6-elimination was faster. However, the difference between the reaction rates of the two eliminations was relatively small. The biphasic kinetics observed for the disassembly of compounds **4** and **5**, is probably derived of the mechanism described in Fig. 2. Both the *para*- and *ortho*-azaquinone-methide eliminations can be used to effectively release drug/reporter molecules under physiological conditions. A similar observation was made previously with mercapto analogs of the corresponding anilines used in this report.[3]

In summary, we have described here a study of a new system that can disassemble through *para*- and *ortho*-azaquinone-methide elimination.[12] The disassembly was evaluated with molecules that undergo single 1,6- or 1,4-elimination and with molecules that undergo double-elimination. The 1,6-elimination was shown to occur slightly faster than the 1,4-elimination under physiological conditions. This study will aid in design of prodrug systems that are based on a stimulus activation and release mechanism.

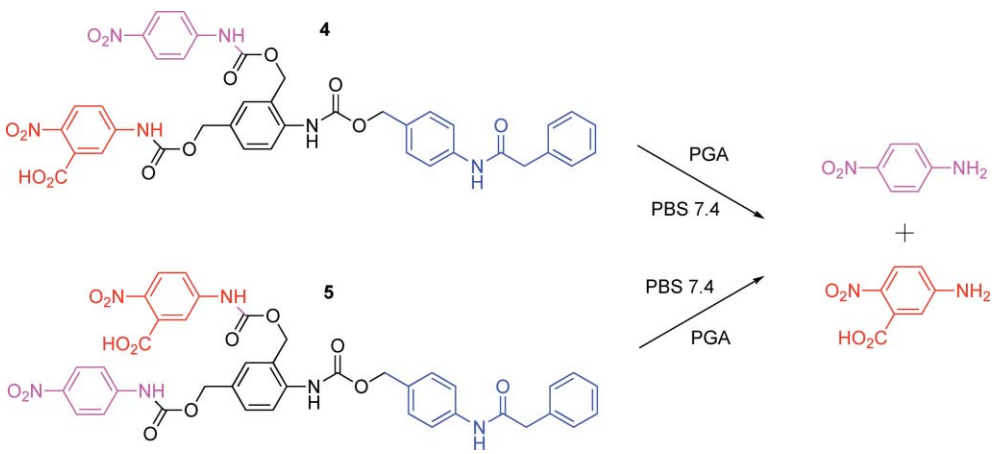

Fig. 5 PGA-catalyzed the release of 5-amino-2-nitrobenzoic acid and 4-nitroaniline from compounds **4** and **5** through a 1,6- and 1,4-double elimination.

Acknowledgements

D.S. thanks the Israel Science Foundation (ISF) and the Binational Science Foundation (BSF) for financial support.

Notes and references

1 P. L. Carl, P. K. Chakravarty and J. A. Katzenellenbogen, *J. Med. Chem.*, 1981, **24**, 479.
2 F. M. de Groot, E. W. Damen and H. W. Scheeren, *Curr. Med. Chem.*, 2001, **8**, 1093, and references therein.
3 P. D. Senter, W. E. Pearce and R. S. Greenfield, *J. Org. Chem.*, 1990, **55**, 2975.
4 M. L. Szalai, R. M. Kevwitch and D. V. McGrath, *J. Am. Chem. Soc.*, 2003, **125**, 15688.
5 R. J. Amir, N. Pessah, M. Shamis and D. Shabat, *Angew. Chem., Int. Ed.*, 2003, **42**, 4494.
6 R. J. Amir and D. Shabat, *Chem. Commun.*, 2004, 1614.
7 A. Sagi, R. Weinstain, N. Karton and D. Shabat, *J. Am. Chem. Soc.*, 2008, **130**, 5434.
8 K. Haba, M. Popkov, M. Shamis, R. A. Lerner, C. F. Barbas, 3rd and D. Shabat, *Angew. Chem., Int. Ed.*, 2005, **44**, 716.
9 M. Shamis, H. N. Lode and D. Shabat, *J. Am. Chem. Soc.*, 2004, **126**, 1726.
10 A. Sagi, E. Segal, R. Satchi-Fainaro and D. Shabat, *Bioorg. Med. Chem.*, 2007, **15**, 3720.
11 C. A. Claridge, A. Gourevitch and J. Lein, *Nature*, 1960, **187**, 237.
12 While this manuscript was under preparation, a related study, describing a similar system that undergoes 1,6- and 1,4-elimination reactions, was published. However, unlike our, the system analyzed by Warnecke *et al.* was separately activated with a chemical reagent and solubility problems did not allow evaluation of the disassembly under physiological conditions. A. Warnecke and F. Kratz, *J. Org. Chem.*, 2008, **73**, 1546.

