The pyridinone-methide elimination†

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The quinone-methide elimination is a common, efficient methodology used in linkers designed to undergo self-fragmentation. Here, for the first time, we demonstrate this elimination in a pyridine ring system. Under physiological conditions, a compound constructed of a pyridine core, a reporter, and an enzymatic trigger underwent significantly faster 1,4-elimination than its parent compound with a benzene core. In addition, an AB_2 self-immolative dendron based on a pyridine core released its two reporter units upon activation through 1,6- and 1,4 pyridinone-methide elimination reactions, again faster than the analogous benzene system. Increased aqueous solubility was observed with compounds based on pyridine relative to those based on benzene. The pyridinone-methide elimination could be applied as an alternative tool in designing self-immolative linkers for release of active target molecules in an aqueous environment.

The quinone-methide elimination is a powerful and efficient reaction used in the design of linkers with self-immolative capabilities. There are numerous examples in the scientific literature of its use in the synthesis of prodrugs and molecular probes.[1–5] It was shown that 4-hydroxy-benzylalcohol or 2-hydroxy-benzylalcohol can be applied as self-immolative spacers to link a reporter molecule and a triggering unit (Fig. 1). Cleavage of the trigger of compounds **1** or **2** generates phenolate **1a** or **2a**, respectively. Phenolate **1a** undergoes 1,6-elimination to release a reporter group (R) and *p*-quinone-methide **1c**, whereas phenolate **2a** undergoes 1,4-elimination to release the reporter and *o*-quinone-methide **2c**. When the reaction is performed under aqueous conditions, the majority of the released quinone-methide is trapped by water to generate the corresponding hydroxy-benzylalcohol. This linking technique is usually applied to activate a prodrug or to release a reporter unit for diagnostic purposes.

The 1,6- and 1,4-quinone-methide eliminations have been used to achieve multiple-release of end groups in dendritic molecules.[6,7] These eliminations are an effective powerful tool in the activation of self-immolative dendrimers[8–12] and self-immolative polymers.[13–15] Very recently we demonstrated a novel dendritic chain reaction that takes advantage of the quinone-methide eliminations to accomplish unique exponential amplification for diagnostic purposes.[16]

Although there have been numerous reports of quinone-methide eliminations in benzene ring systems, there are no examples of this elimination in a pyridine ring. Pyridine is a water-soluble compound whereas benzene is completely insoluble in aqueous media. Thus, use of linkers with a pyridine moiety for applications to be performed under aqueous conditions should increase the compounds' water solubility. Here we report the first evaluation of the quinone-methide type elimination in a pyridine ring system.

Initially, we decided to explore the disassembly of pyridine system **3** (Fig. 2). This molecule has a phenylacetamide triggering group that, upon cleavage by penicillin-G-amidase[17] (PGA), generates amine **3a**. This amine undergoes spontaneous 1,6-elimination and decarboxylation to release amine **3b**, which then releases **3c** by cyclisation of the side chain to form an *N*,*N*′-dimethylurea. This intermediate should then undergo 1,4-elimination to release the reporter and the *o*-pyridinone-methide.

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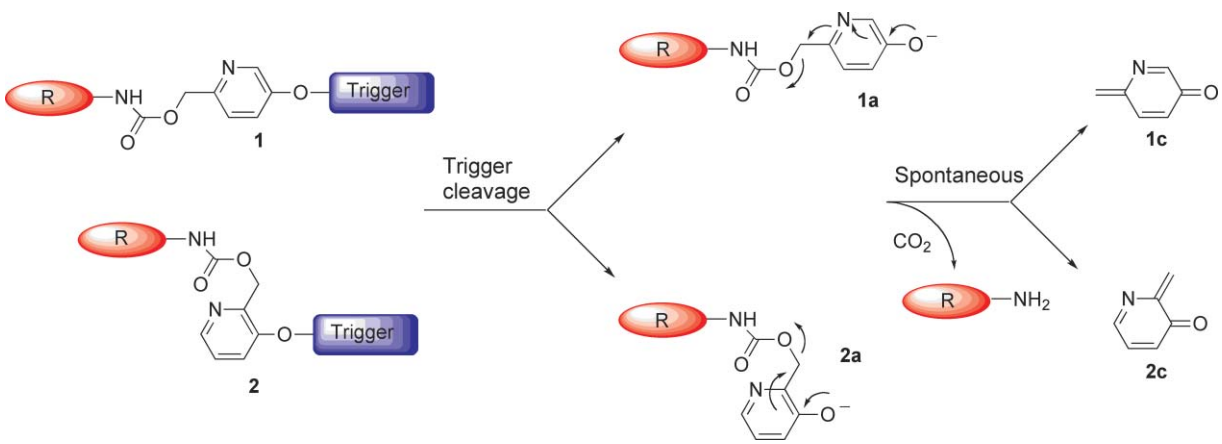

Fig. 1 The 1,6- and 1,4-quinone-methide elimination pathways. R could be a reporter unit used for diagnostic purposes or a drug molecule.

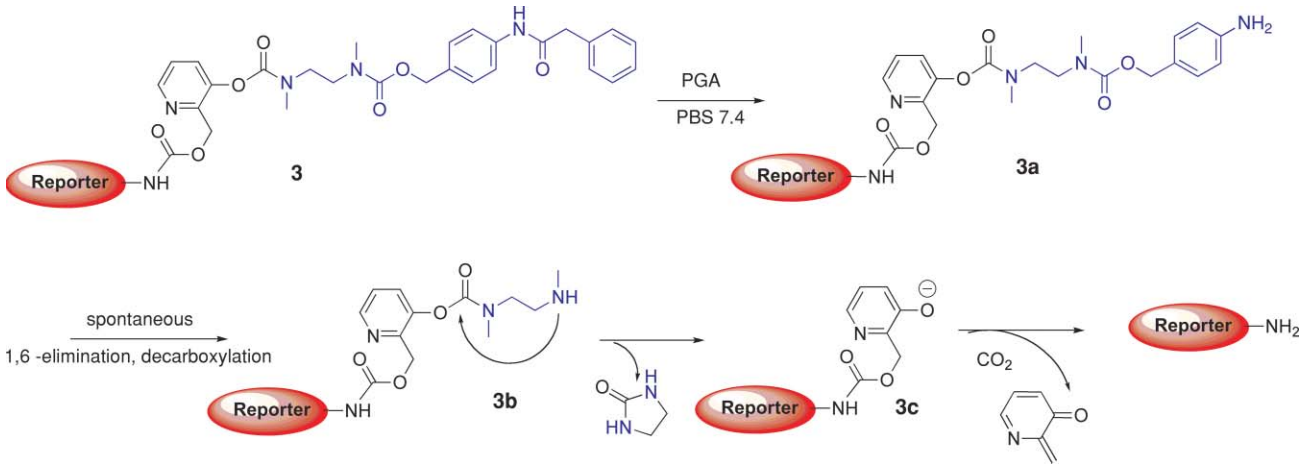

Fig. 2 PGA-triggered 1,4-quinone-methide elimination in a pyridine ring system.

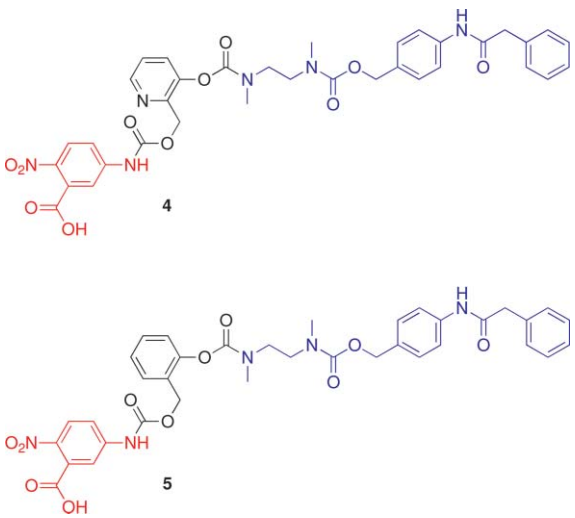

Fig. 3 Chemical structures of compounds designed to undergo 1,4-quinone-methide elimination (compound **4**, pyridine system; compound **5**, benzene system).

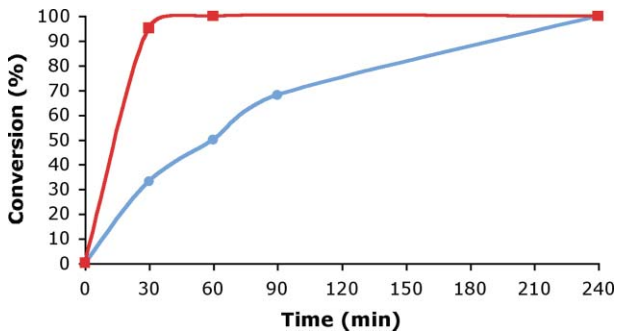

Fig. 4 PGA-catalyzed release of 5-amino-2-nitrobenzoic acid from compound **4** (-▩-), [100 μM], ($Kobs = 3.2 \times 10^{-2}$) and from compound **5** (-◍-), [100 μM], ($Kobs = 0.41 \times 10^{-2}$) in PBS, 25 °C monitored by RP-HPLC. Enzyme concentration was 0.1 mg/ml.

Since we sought to compare the 1,4-quinone-methide elimination in pyridine to that of benzene, we synthesized compounds **4** and **5** (see ESI†). Both contained a phenylacetamide trigger and 5-amino-2-nitrobenzoic acid reporter group (Fig. 3). Compound **4** was constructed with a pyridine system, whereas compound **5** incorporated a benzene system.

Compounds **4** and **5** were incubated in phosphate buffered saline (pH 7.4; PBS) in the presence of PGA and the release of 5-amino-2-nitrobenzoic acid was monitored by RP-HPLC (Fig. 4). The pyridine system **4** disassembled significantly faster than benzene system **5**. While four hours were needed in order to complete the release of the reporter from compound **5**, only 30 min were required for compound **4** to complete the release. No reporter release was observed in the absence of PGA (data not shown).

With these results in hand, we evaluated the disassembly of a more complex system (Fig. 5). Molecule **6** is an AB_2 type self-immolative dendron that has a phenylacteamide trigger and two reporter units attached to a pyridine core. The compound was designed to release phenolate **6a** upon reaction with PGA. The latter undergoes a first elimination to release one reporter and *p*-pyridinone-methide **6b**, which can then react with a water molecule to generate phenolate **6c**. This phenolate undergoes a second elimination to release the other reporter and *o*-pyridinone-methide **6d**. We have previously shown that the *p*-quinone-methide elimination occurs to some extent faster than the *o*-quinone-methide elimination.[9]

In order to evaluate the double pyridinone-methide eliminations in comparison to analogous eliminations in a benzene ring system, we synthesized compounds **7** and **8** (see ESI†). Both are equipped with phenylacetamide trigger and two units of 5-amino-2-nitrobenzoic acid as reporter groups (Fig. 6). Compound **7** was constructed with a pyridine core, whereas compound **8** has a benzene core.

As in the previous experiment, compounds **7** and **8** were incubated in PBS in the presence of PGA and the release of 5-amino-2-nitrobenzoic acid was monitored by RP-HPLC (Fig. 7). The obtained results were correlated with the single elimination release data presented in Fig. 4. The pyridine system **7** disassembled significantly faster than benzene system **8**. While 9 h were needed in order to complete the release of the reporters from compound **8**, less than 60 min were required for compound **7** to complete the release. No reporter release was observed in the absence of PGA (data not shown).

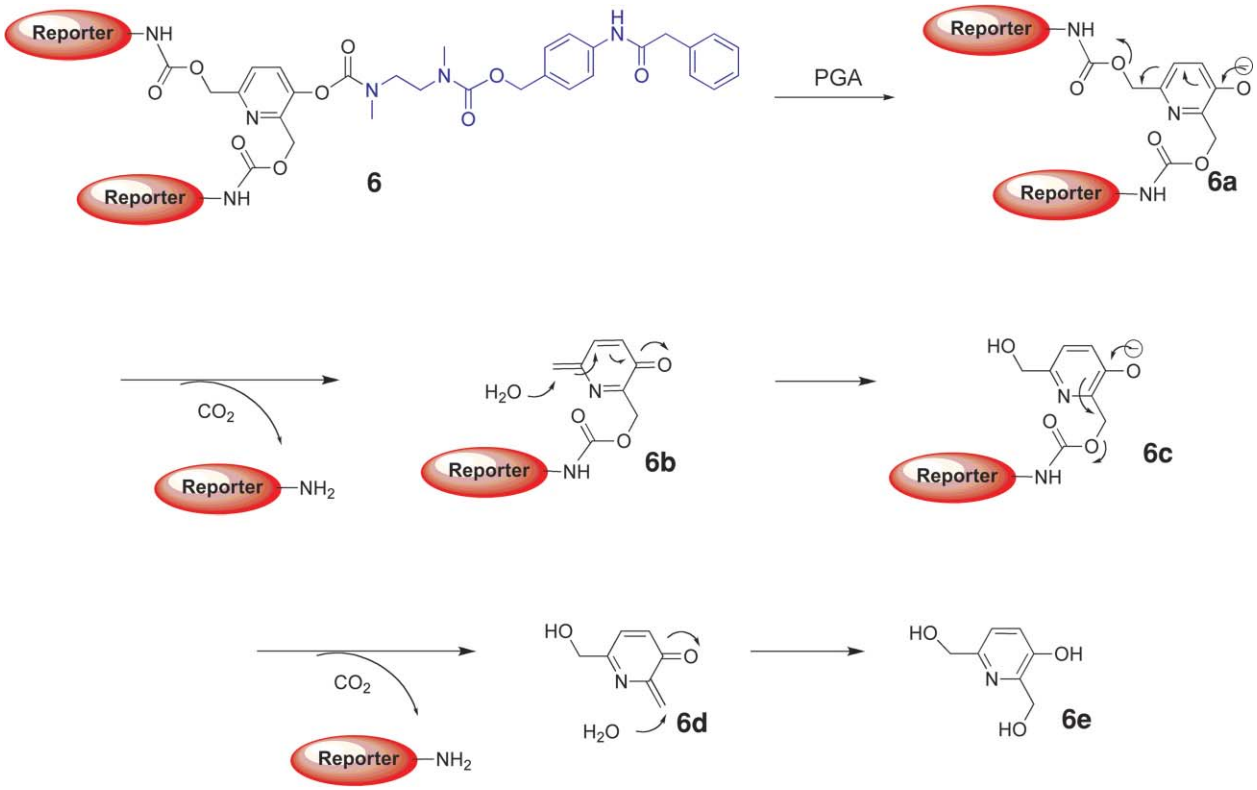

Fig. 5 Disassembly pathway of self-immolative dendron **6** through double pyridinone-methide eliminations to release two reporter units.

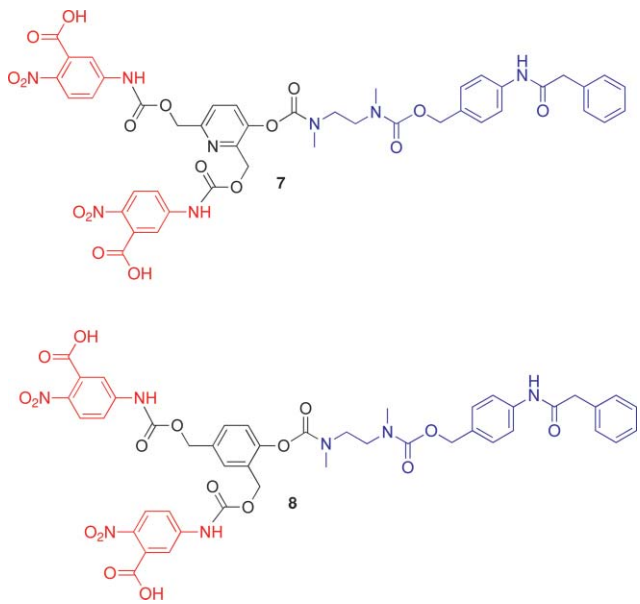

Fig. 6 Chemical structures of dendritic compounds designed to undergo double quinone-methide type eliminations (compound **7**, pyridine system; compound **8**, benzene system).

Pyridine-based compounds **4** and **7** underwent pyridinone-methide eliminations more rapidly than their benzene-based counterparts. In order to facilitate such elimination, a phenolate precursor must initially be formed (intermediate **3c** in Fig. 2 and intermediates **6a** and **6c** in Fig. 5). Since the pKa of 3-hydroxypyridine (8.7) is more acidic than that of phenol (10.0) under aqueous conditions, the relative concentration of the formed

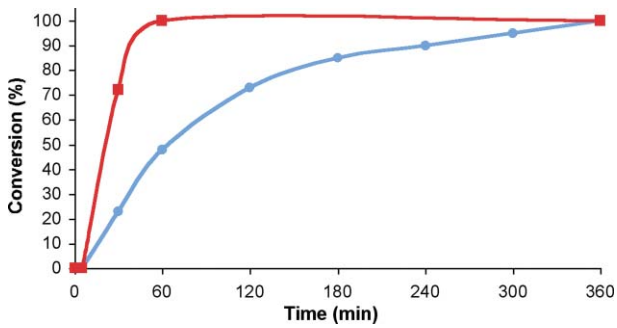

Fig. 7 PGA-catalyzed release of 5-amino-2-nitrobenzoic acid from compound **7** (-▩-), [100 μM], ($Kobs = 2.1 \times 10^{-2}$) and from compound **8** (-◍-), [100 μM], ($Kobs = 0.28 \times 10^{-2}$) in PBS, 25 °C monitored by RP-HPLC. Enzyme concentration was 0.1 mg/ml.

phenolate is expected to be greater in the pyridine system than in the benzene system. The difference in kinetics of the quinone-methide type elimination observed in the pyridine ring systems in comparison to benzene systems can be explained by this pKa difference.

In this study, we used compounds with reporter units of 5-amino-2-nitrobenzoic acid. This unit contains an ionizable carboxylic acid, which generates aqueous solubility for both pyridine- and benzene-based compounds. However, when we evaluated compounds with a 4-nitroaniline reporter unit, which does not have the ionizable carboxylic acid, only molecules with the pyridine core could be activated by PGA (data not shown). Compounds with the benzene core were completely insoluble in water and, therefore, could not be evaluated for enzymatic activation and elimination reactions.

In conclusion, a system constructed of a pyridine core, a reporter, and an enzymatic trigger underwent more rapid 1,4-elimination under physiological conditions than its parent system with a benzene core. Furthermore, an AB_2 self-immolative dendron based on a pyridine core released its two reporter units upon activation through 1,6- and 1,4 pyridinone-methide elimination reactions, again, faster than the analogous benzene system. Increased aqueous solubility was observed with compounds based on pyridine rather than benzene. This study should aid the design of new self-immolative linkers for prodrugs[18] or other applications requiring linkers that are cleavable under aqueous conditions.

Acknowledgements

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