Self-immolative dendrimer biodegradability by multi-enzymatic triggering†

Roey J. Amir and Doron Shabat*

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

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New self-immolative dendritic molecules have been designed and synthesized. The dendrons are built with a multi-enzymatic triggering mechanism, which initiates their biodegradation through a self-immolative chain fragmentation to release a reporter group from the focal point. The dendritic backbone is constructed from polycarbamate linkages, which are stable to hydrolysis and enhance the dendrons' solubility in water. The degradation can readily take place under physiological conditions on enzymatic triggering.

Degradable dendrimers have been attracting special interest in the scientific community.1−2 They are particularly desirable in the field of controlled drug delivery systems;3−5 after a dendritic platform has ended its task as a drug carrier it needs to be cleared out from circulation. Biodegradability of a dendrimer should speed up its clearance and avoid undesired toxicity side effects.7,8 At this time, there are only a few known examples of dendrimers that degrade by controlled fragmentation.1,9 Recently, we and others have introduced a new class of dendritic molecules which were termed self-immolative dendrimers.10−12 These structurally unique dendrimers can release all of their tail units, through a self-immolative mechanism which initiates the domino breakdown that will release the reporter group. Im- portantly, only one enzymatic cleavage out of a possible four is sufficient to initiate the domino breakdown that will release the reporter group at the focal point of the dendrimer. The complete degradation of the dendron to its building blocks is depicted in Scheme 3.

The dendritic molecules were prepared straightforwardly as shown in Scheme 4 and the ESI.† Thus, dendron 1 was obtained by reaction of phenylacetamide chloride with mono-Boc-N-methyl-ethylendiamine to afford compound 4, followed by Boc removal and addition of dinitrophenyl carbonate. Dendron 2 was prepared by reaction of diethylentriamine with imidazol amide of phenylacetic acid to afford compound 5, which was further reacted with dinitrophenyl carbonate. Coupling of compound 5 with active carbonate of 4-hydroxy-benzylalcohol afforded alcohol 6, which was further activated with 4-nitrophenylchloroformate to give compound 7. The latter (two equivalents) was reacted with diethylentriamine and a subsequent one pot reaction with dinitrophenyl carbonate afforded dendron 3.

Dendrons 1–3 were incubated with PGA in PBS pH 7.4 at 37 °C. Their biodegradation could be conveniently monitored by following the formation of 4-nitrophenol with visible spectroscopy at a wavelength of 405 nm. The kinetic release of 4-nitrophenol from the dendrons is shown in Fig. 1. Upon addition of PGA to dendrons 1–3, free 4-nitrophenol was gradually formed, indicating that PGA cleaves its phenylacetamide substrate and the degradation indeed occurs as was predicted. As we expected the G1-dendron released...
the 4-nitrophenol faster than the G0-dendron while the G2-dendron released it relatively more slowly. The background control reactions showed no release at all.

The kinetic constants $K_{obs}$ for the three reactions were calculated by linear correlation with the measured plots (Table 1). The phenomenon of dendron 2 releasing its reporter group faster than dendron 1 occurs since the enzymatic substrate concentration in dendron 2 is twice as high as in dendron 1. The following self-cyclization step is relatively fast and therefore, the rate-limiting step is cleavage of the enzymatic substrate. In dendron 3 additional self-immolative reactions occur in order to complete the release of the reporter group (another intra-cyclization and 1,6-quinone-methide elimination). The overall rate of these reactions is slower than the rate of the enzymatic substrate cleavage and therefore the $K_{obs}$ for dendron 3 is relatively smaller.

In conclusion, we have designed and synthesized new dendritic molecules with a multi-enzymatic triggering mechanism that initiates their biodegradation through a self-immolative chain fragmentation to release a reporter group from the focal point. For the first time, the potential of diethylenetriamine was introduced as a double trigger linker, which can be used as a building block for constructing self-immolative dendrimers. The dendrons were found to have fairly good (G0, G1) to moderate (G2) water solubility and high stability to background hydrolysis under physiological conditions. Their degradation readily occurs in aqueous medium and can easily be monitored by generation of free reporter molecule. Incorporation of different substrates on the dendron’s periphery should allow the use of varying triggering enzymes. 17 This concept may be particularly important in the field of prodrug mono-therapy,18 if a drug molecule will be incorporated instead of the reporter unit, 19 especially in circumstances with more than one tumor-associated or targeted enzyme with different catalytic activity. Further studies of these dendritic molecules are under progress.

Notes and references


### Table 1

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<th>Dendron 1</th>
<th>Dendron 2</th>
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<td>$K_{obs}$/min$^{-1}$</td>
<td>5.11</td>
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