NJC

Cite this: *New J. Chem.*, 2012, **36**, 386–393

www.rsc.org/njc

PAPER

Exponential diagnostic signal amplification *via* dendritic chain reaction: the dendritic effect of a self-immolative amplifier component†‡

Naama Karton-Lifshin and Doron Shabat*

Received (in Victoria, Australia) 6th June 2011, Accepted 27th August 2011
DOI: 10.1039/c1nj20486f

Signal amplification techniques are commonly used to boost diagnostic signals. We have recently developed a new approach for exponential signal amplification obtained through a distinctive dendritic chain reaction. Here we report evaluation of the effect of the self-immolative dendritic amplifier component on the signal amplification. Four dendrons with various numbers of end-units were evaluated for the ability to produce exponential signal amplification through self-immolative disassembly pathways. The dendron composed of a first-generation platform with three end-units exhibited the best characteristics with rapid disassembly rate and good stability under aqueous conditions. This study demonstrates the efficiency of molecules based on dendritic structures.

Introduction

Development of new signal amplification techniques is needed in order to improve the ability to detect trace amounts of a wide variety of analytes. Therefore, there is a considerable interest in the development of new molecularly based amplification methodologies.[1–10] Recently, we reported on a new distinctive molecular system that generates exponential signal amplification through a dendritic chain reaction (DCR).[11,12] The system is based on the unique disassembly properties of self-immolative dendrimers, where a single stimulus event by a specific analyte at the dendron focal point is translated into release of multiple peripheral end units.[13–15] When the end units are released, they acquire the chemical reactivity of the analyte of interest and thereby can then activate additional dendrons to produce a chain reaction that progresses through an exponential disassembly mode. The system disassembly is monitored by a reporter molecule, which is also released from the self-immolative dendron to produce exponential signal growth. A simpler version of the DCR system was later developed, in which a separated chromogenic probe and a self-immolative dendron were used together to produce exponential signal amplification through a two-component dendritic chain reaction (2CDCR).[16] The modular design of the DCR probes allows introduction of detection capabilities for various compounds through incorporation of a trigger that is reactive with the analyte of interest. Thus far, this strategy has been demonstrated for detection of two different analytes: hydrogen peroxide and ubiquitous sulfhydryl compounds.[17] In the 2CDCR approach the self-immolative dendron is used as an amplifier moiety and the other component as the signal generator. Hence, the rate of amplification should in part depend on the number of end-groups on the self-immolative dendron. Here we report an evaluation study of the dendritic effect on the signal produced by the 2CDCR technique.

To evaluate the dendron-generation effect on the 2CDCR, we chose a model system designed for detection of hydrogen peroxide (Fig. 1). Dendron **1** is composed of two glucose units and a phenylboronic-acid trigger, which is cleaved upon reaction with hydrogen peroxide.[5] Probe **2** is composed of the reporter 5-amino-2-nitrobenzoic acid attached to the phenylboronic-acid trigger. The two-component DCR amplification cycle of dendron **1** and probe **2** is illustrated in Fig. 1. Cleavage of the trigger of dendron **1** by a hydrogen peroxide molecule will release two glucose molecules. The two free glucose units will be oxidized by glucose oxidase (GOX) present in solution to produce two molecules of hydrogen peroxide, which then activate additional dendrons. The rate of disassembly should exponentially increase until all the reporter molecules have been released. The signal can be detected with a spectrophotometer by monitoring the yellow color of the released 5-amino-2-nitrobenzoic acid.

Glucose is used as a precursor for hydrogen peroxide since it can be easily oxidized by the commercially available enzyme GOX after its release from the dendritic platform. In addition, the hydrophilicity of glucose increases the aqueous solubility of the relatively lipophilic dendrons.

Results and discussion

In order to employ the proposed dendritic chain reaction for detection of hydrogen peroxide, two major chemical

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: Full experimental details, characterization data of all new compounds (PDF). See DOI: 10.1039/c1nj20486f

‡ This article is part of the themed issue Dendrimers II, guest edited by Jean-Pierre Majoral

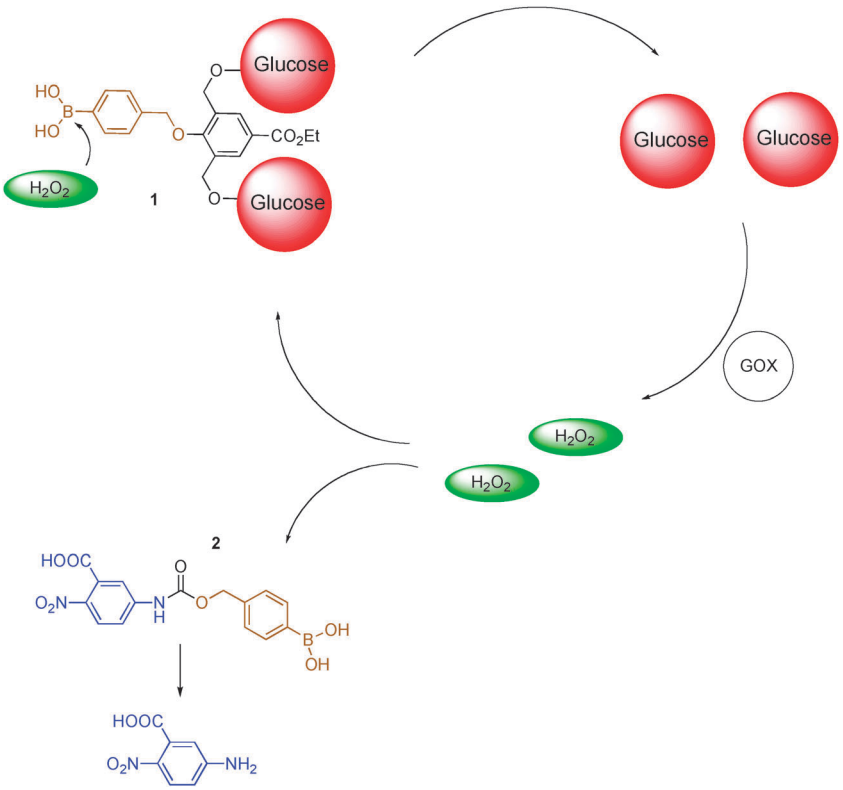

Fig. 1 A two-component DCR system to detect hydrogen peroxide; glucose reagent units and 5-amino-2-nitrobenzoic acid reporter are indicated.

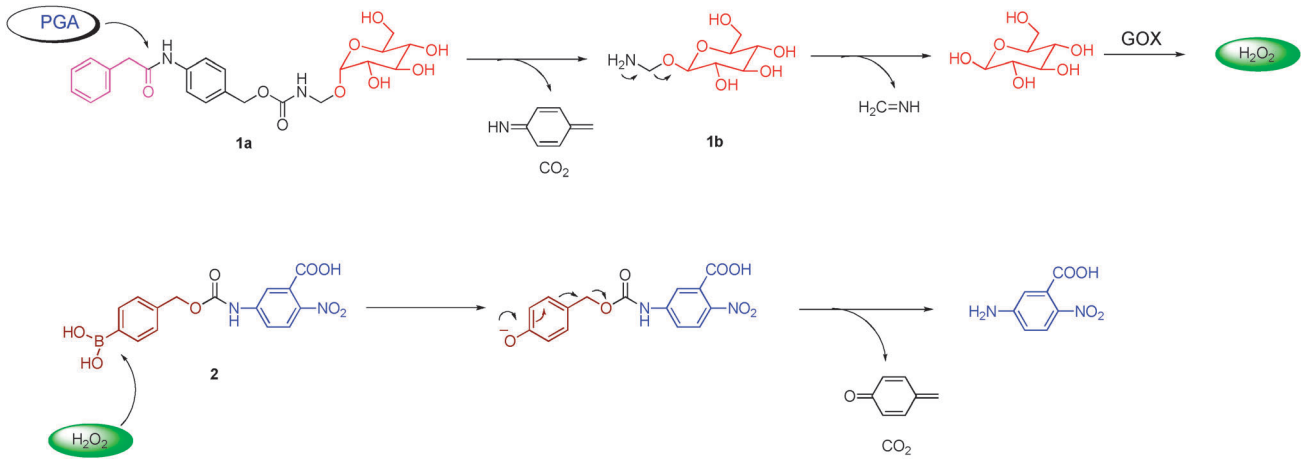

Fig. 2 Disassembly mechanism of compound **1a** and probe **2**. PGA-responsive release of covalently linked glucose to generate hydrogen peroxide is shown.

reactivities must be in hand: a "disassembly" chemical pathway that enables the release of a covalently linked glucose and a specific protecting group that is cleaved by a reaction with hydrogen peroxide. These two reactions are illustrated in Fig. 2. The release of a covalently linked glucose was achieved through the design of molecules like compound **1a** (see ESI† for synthesis). This compound is composed of a glucose molecule linked through a self-immolative linker to phenylacetamide—a substrate for penicillin-G-amidase (PGA). When PGA, a model-cleavage reagent, was incubated with the compound **1a**, the generated aniline undergoes 1,6-elimination to release intermediate **1b**, which is further disassembled to release the glucose molecule. The free glucose is then oxidized by GOX, also present in the solution, to form hydrogen peroxide, which then can activate probe **2** to release the 5-amino-2-nitrobenzoic acid reporter.

Probe **2** and model-compound **1a** were incubated in PBS (pH 7.4), in the presence of GOX with or without PGA. The release of the 5-amino-2-nitrobenzoic acid was monitored by UV-Vis spectroscopy. As expected, in the presence of PGA and compound **1a**, the reporter was released from probe **2**; no release was observed in the absence of the enzyme (Fig. 3). The proposed disassembly pathway clearly occurred, and release of a covalently linked glucose produced hydrogen peroxide, which activated a phenylboronic acid protecting group-based probe.

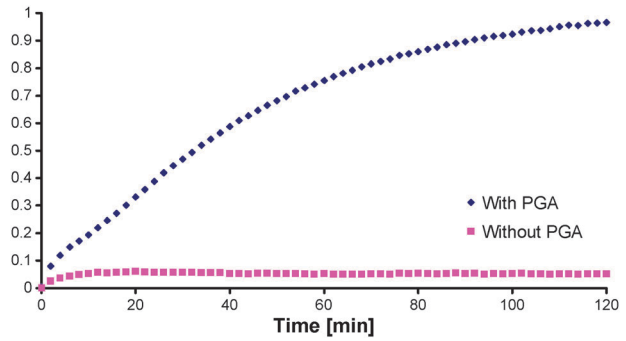

Fig. 3 PGA-catalyzed release of 5-amino-2-nitrobenzoic acid through sequential disassembly of compounds **1a** and **2**. Conditions: wavelength, 405 nm; **1a** and **2** [500 μM] in PBS (pH 7.4) and PGA [0.01 mg mL^{-1}]; 25 °C.

Four different self-immolative dendrons were synthesized, each equipped with a phenylboronic-acid as a trigger with various numbers of glucose end-units. Dendrons **3** (AB_2, attached to two glucose units) and **4** (AB_3, attached to three glucose units) are based on a first-generation dendritic platform, whereas dendrons **5** (AB_4, attached to four glucose units) and **6** (AB_6, attached to six glucose units) are based on a second-generation dendritic platform (Fig. 4).

Dendrons **3**, **4**, **5** and **6** were synthesized by linking the glucose units through carbamate linkages generated *in situ* from acylazide **9** (Fig. 5). The synthesis of molecule **9** was performed by coupling of compound **7**[18] with *N*-hydroxy-succinimide (NHS) generated NHS-ester derivative **8**. The latter was reacted with sodium azide to afford acylazide **9**.

Next, we synthesized AB_2 dendron **3** that contains two glucose units and phenylboronic acid as a trigger unit.

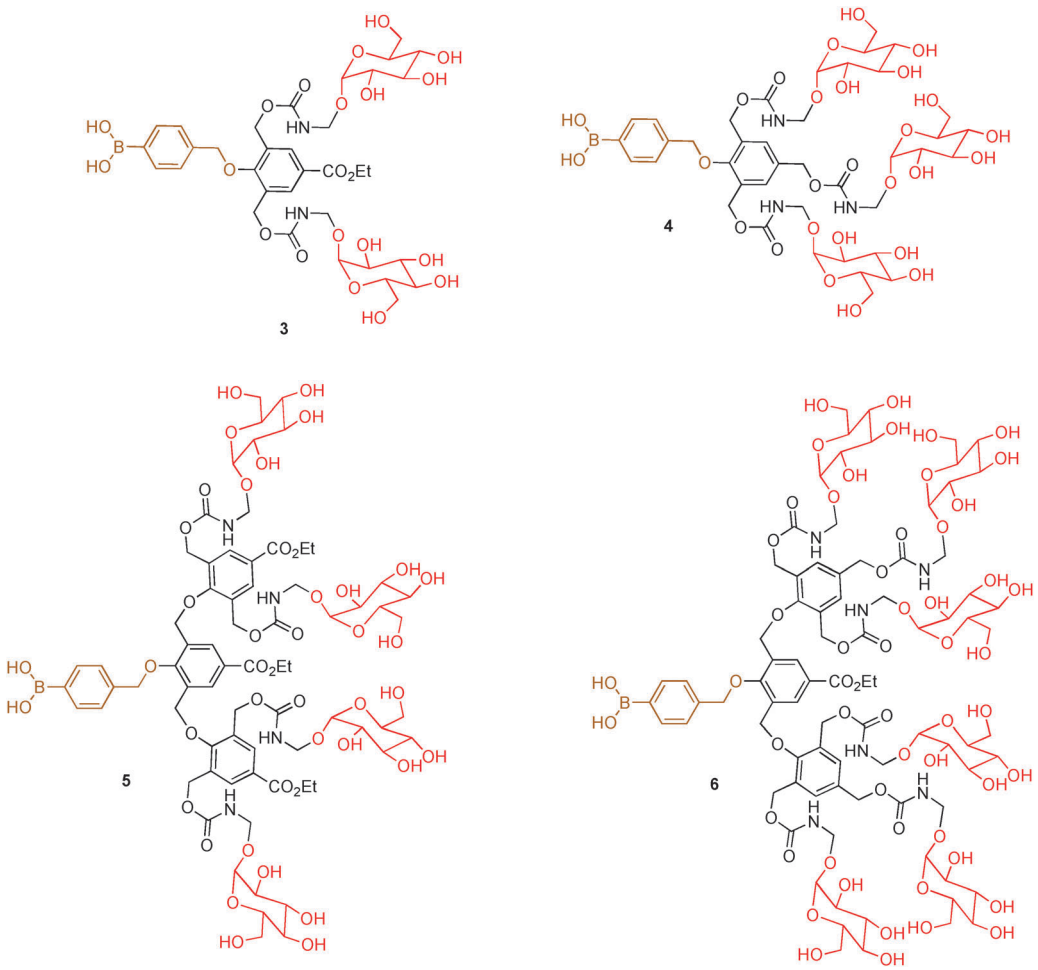

Fig. 4 Chemical structures of self-immolative dendrons equipped with a phenylboronic-acid trigger and various numbers of glucose end-units.

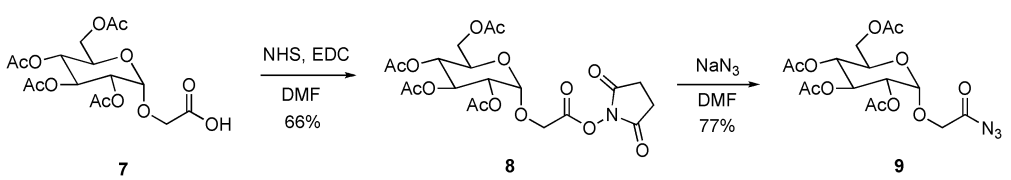

Fig. 5 Chemical synthesis of glucose derivative **9**.

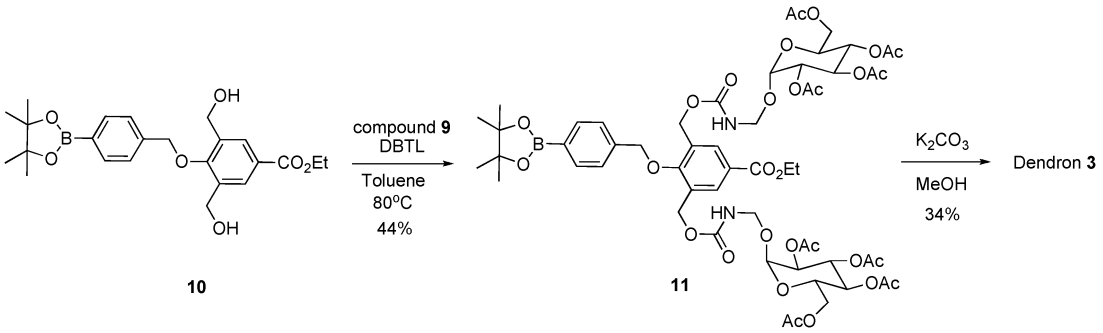

Fig. 6 Chemical synthesis of self-immolative AB_2 dendron **3**.

Synthesis of compound **3** was performed according to the synthetic strategy presented in Fig. 6. Curtius rearrangement of four equivalents of acylazide **9** generated an isocyanate, which was reacted *in situ* with diol **10**[12] to afford compound **11**. Deprotection of the acetate groups using a catalytic amount of potassium carbonate in methanol afforded AB_2 dendron **3**.

The AB_3 dendron was synthesized using a procedure similar to that described for the AB_2 system (Fig. 7). Dendron **12**[16] was treated with *p*-TsOH to afford triol **13**. The latter was reacted with six equivalents of compound **9** to give the protected form of dendron **14**. Deprotection of the acetate groups afforded AB_3 dendron **4**.

The synthesis of AB_4 dendron **5** was performed according to the strategy shown in Fig. 8. Iodination of diol **10** with sodium iodide and chlorotrimethylsilane generated benzyliodide derivative **15**. Etherification of the latter with compound **16**[12] gave dendron **17**. The *tert*-butylsilyl protecting groups were then removed using a catalytic amount of *p*-TsOH to afford dendron **18**, which was further treated with eight equivalents of compound **9** to yield compound **19**. Deprotection of the acetate groups as previously described afforded dendron **5**.

Synthesis of AB_6 dendron **6** was achieved as presented in Fig. 9. Etherification of phenol **20** by using benzyliodide derivative **15** and potassium carbonate gave dendron **21**. Removal of the *tert*-butylsilyl protecting groups afforded dendron **22** and further reaction with eight equivalents of compound **9** gave compound **23**. Deprotection of the acetate groups as previously described afforded dendron **6**.

With four self-immolative dendrons in hand, we evaluated the ability of the first dendron (AB_2 platform) to produce a two-component DCR reaction. Dendron **3** and probe **2** were incubated with various amounts of hydrogen peroxide in the presence of GOX and the release of the 5-amino-2-nitro-benzoic acid reporter was monitored at a wavelength of 405 nm (Fig. 10).

When 1.0 equivalent of hydrogen peroxide (*vs.* dendron **3**) was used, the system reached complete disassembly within 50 min. As expected, disassembly was slower when less hydrogen peroxide was used (Fig. 10). The exponential progress of the system disassembly is demonstrated by the sigmoidal plots obtained for reaction with various equivalents of H_2O_2. The background signal obtained due to some spontaneous hydrolysis was also amplified; therefore, the sensitivity of this system for detection of H_2O_2 is limited to the low μM range. As described for dendron **3**, we evaluated the ability of dendrons **4**, **5** and **6** to produce a two-component DCR reaction. As expected, all dendrons disassembled to produce exponential signal growth upon incubation with H_2O_2, although rates differed (see ESI†).

The 2CDCR disassembly behaviors of the self-immolative dendrons were then compared at the same concentration of H_2O_2. Due to the higher number of glucose units released in

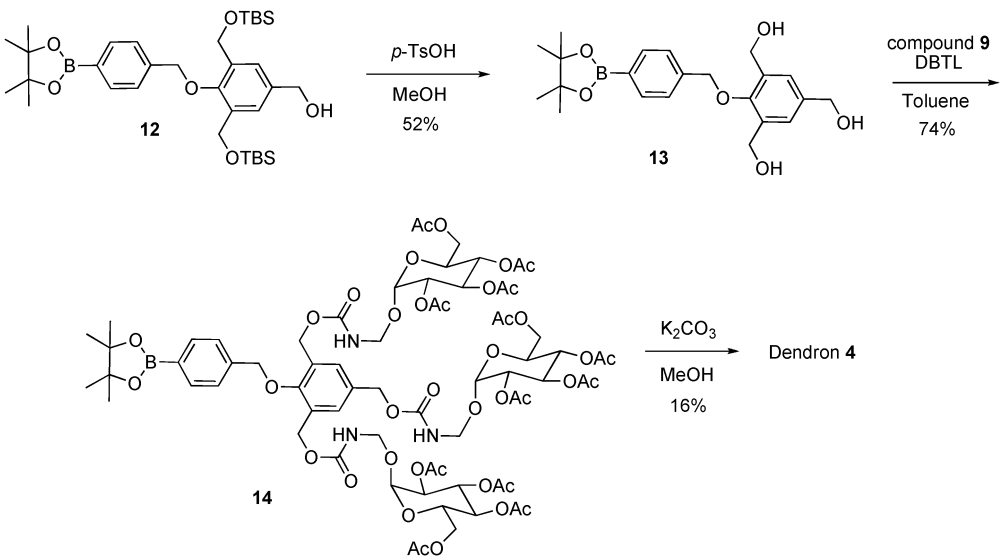

Fig. 7 Chemical synthesis of self-immolative AB_3 dendron **4**.

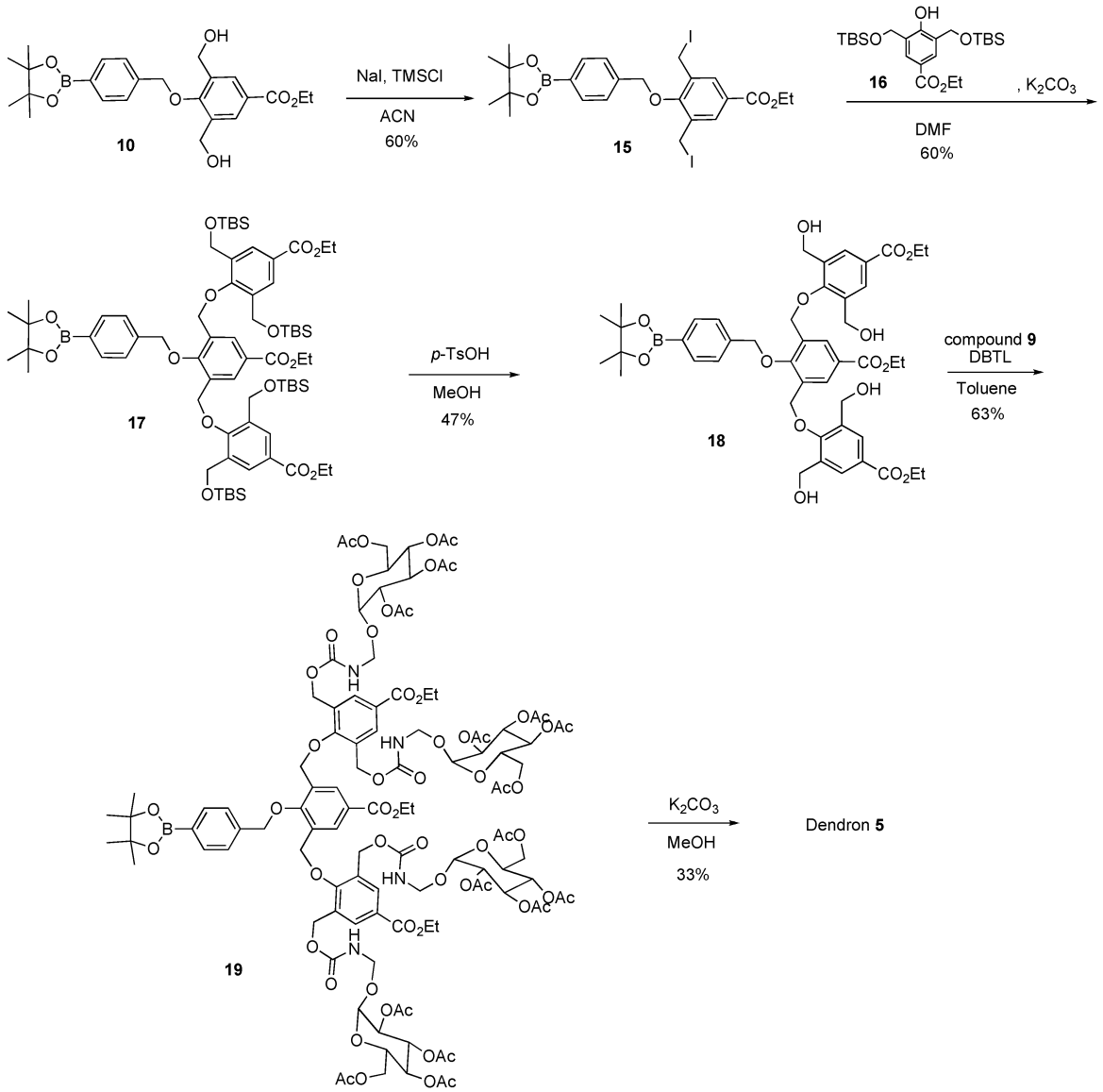

Fig. 8 Chemical synthesis of self-immolative AB_4 dendron **5**.

each disassembly cycle, AB_6 dendron **6** had the fastest amplification rate, whereas the AB_2 dendron **3** was the slowest (Fig. 11). The amplification rate observed for AB_4 dendron **5**, despite release of a higher number of glucose units, was slower than that produced by AB_3 dendron **4**. A possible explanation for this phenomenon could lie in the number of elimination reactions that occurred during the release of the glucose units from the dendrons. AB_3 dendron **4** is based on a first-generation dendritic platform, which undergoes two 1,4 eliminations and one 1,6 elimination in order to release three glucose units. AB_4 dendron **5** is based on a second-generation dendritic platform, which undergoes six 1,4 eliminations in order to release four glucose units. AB_6 dendron **6** is based on a second-generation dendritic platform that undergoes the highest number of elimination reactions (in comparison to the other dendrons) in order to release its glucose units. However, since in every disassembly cycle six glucose units are released, this dendron had the highest amplification rate for the 2CDCR systems tested.

The disassembly behaviors of each of the four dendrons were also compared with 0.1 equivalents and 0.01 equivalents of H_2O_2. The amplification effect increased as the hydrogen peroxide concentration decreased. This observation further demonstrates the capability of the 2CDCR amplification effect, which is more significant at lower analyte concentrations.

In order to determine the most efficient system for the 2CDCR amplification, we also sought to examine the hydrolytic stability of our dendrons. Disassembly of the self-immolative dendrons as a result of hydrolysis generated a background reaction that decreased the signal-to-noise ratio of the probe system. For example, although the AB_6 dendron had the highest rate of disassembly, its hydrolytic stability may be relatively low due to the high number of chemical bonds that can undergo spontaneous hydrolysis. To compare the signal-to-noise ratios of the different 2CDCR systems, the background noise was subtracted from the measured signal and the obtained net-signal was plotted as a function of time for the same hydrogen peroxide concentration (Fig. 12).

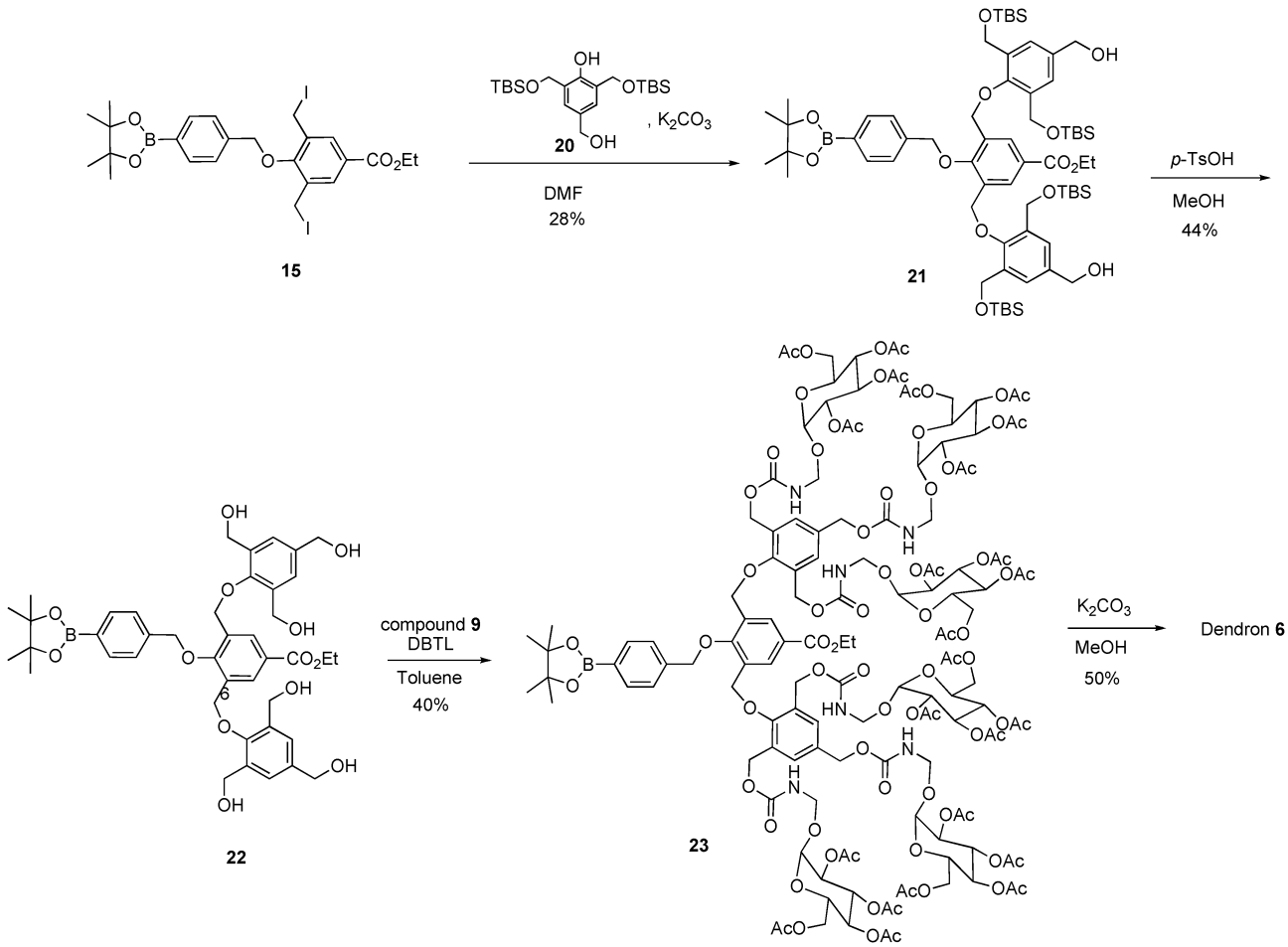

Fig. 9 Chemical synthesis of AB_6 dendron **6**.

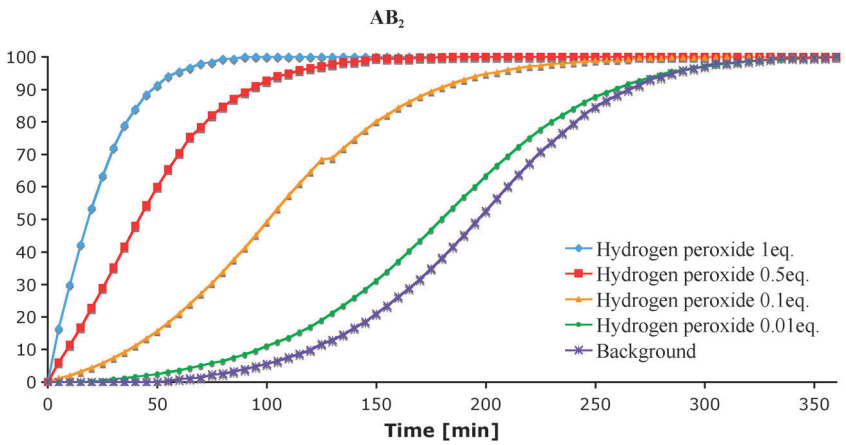

Fig. 10 The release of 5-amino-2-nitrobenzoic acid from probe **2** [500 μM] in the presence of dendron **3** [1000 μM] and GOX [0.1 mg mL^{-1}] in PBS (pH 8.3) upon addition of buffer only (background) or the indicated equivalents of H_2O_2. The reaction progress was monitored at 25 °C, a wavelength of 405 nm for the indicated time period.

Dendrons that disassemble more rapidly are expected to exhibit curve-maximum (T_{max}) at shorter time scales. Indeed, the curve produced by AB_6 dendron **6** had the shortest T_{max}, whereas the curve produced by AB_2 dendron **3** was the longest. AB_3 dendron **4** had a faster disassembly rate than AB_4 dendron **5** and this phenomenon is reflected in the T_{max} values. An additional desirable characteristic for a probe system with a large signal-to-noise ratio is derived from the value of the net-signal. Dendrons with better hydrolytic stability are expected to exhibit larger values of net-signal. The AB_3 dendron **4** seems to be the

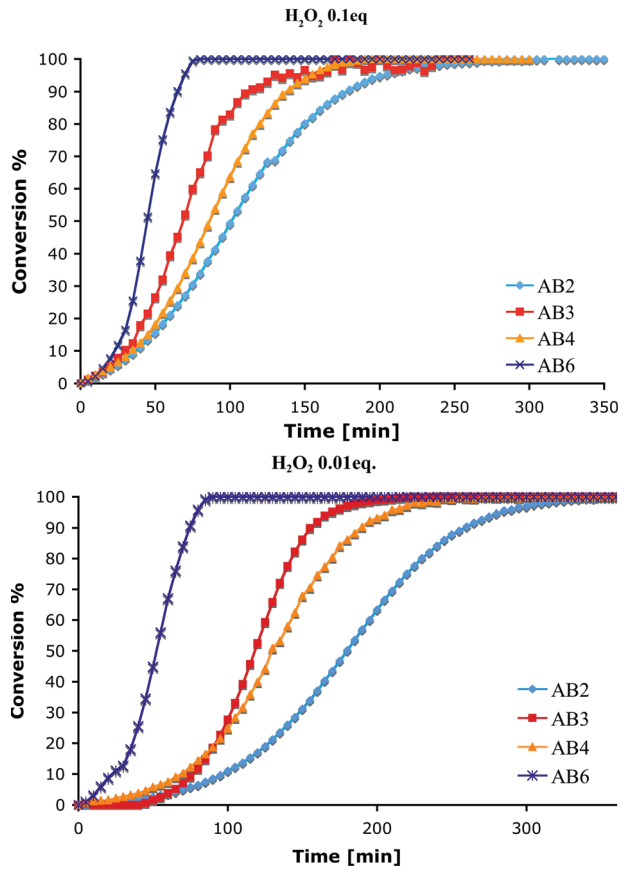

Fig. 11 Comparison of signals measured due to the release of 5-amino-2-nitrobenzoic acid from probe **2** [500 μM] in the presence of the indicated dendrons [1000 μM] and GOX [0.1 mg mL^{-1}] in PBS (pH 8.3) upon incubation with 0.1 and 0.01 equivalents of H_2O_2. The reaction progress was monitored at 25 °C, a wavelength of 405 nm for the indicated time period.

ideal component for the 2CDCR system based on these characteristics. This dendron exhibited a high hydrolytic stability with a relatively fast disassembly rate, which is expressed in its T_{max} value.

Measuring of dendritic effects in the 2CDCR system further supported the amplification advantage observed for AB_3 dendron **4**. The concentration of each dendron was calibrated to the obtained identical released glucose units in each 2CDCR system and the disassembly rate was measured for a fixed hydrogen peroxide concentration (Fig. 13).

Under these conditions, in the absence of dendritic effects, all dendrons are expected to exhibit similar disassembly behavior. As observed in Fig. 11, AB_4 dendron **5** showed a negative dendritic effect, whereas AB_3 dendron **4** had a more positive dendritic effect than AB_2 dendron **3**. The negative dendritic effect observed for the AB_4 dendron can be explained by the ratio between the numbers of elimination reactions that occur in each disassembly cycle and the number of glucose units that are released. The AB_4 dendron undergoes three more elimination reactions than does the AB_3 dendron; however, only one more glucose unit is released in each disassembly cycle. The AB_4 dendron undergoes two less elimination reactions than the AB_6 dendron, but also two less glucose units are released in each disassembly cycle.

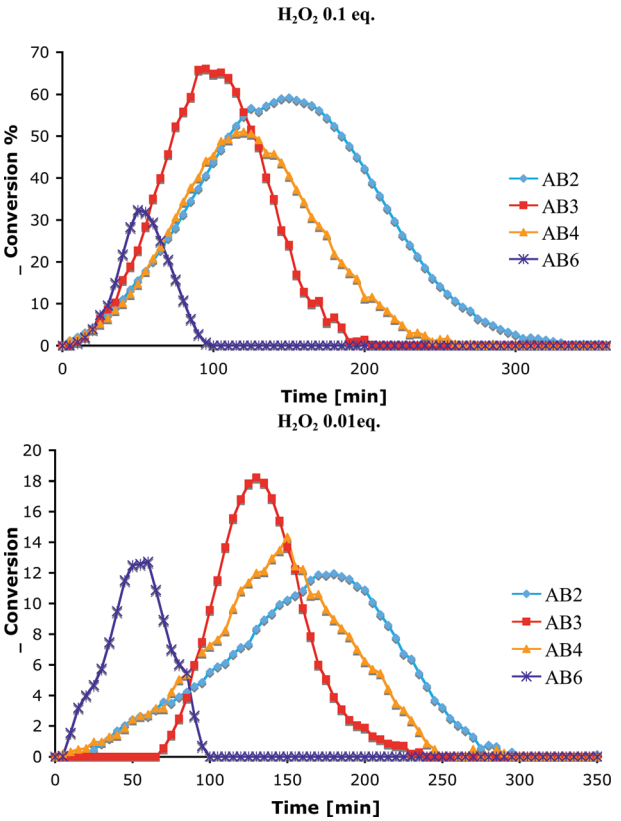

Fig. 12 Comparison of signals measured in the presence of the indicated dendrons *vs.* time. The background signal observed as a function of time was subtracted from the measured values of the corresponding 2CDCR systems.

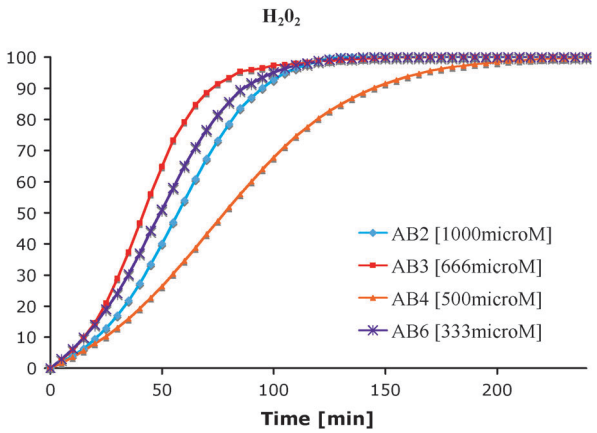

Fig. 13 Comparison of signals measured due to the release of 5-amino-2-nitrobenzoic acid from probe **2** [500 μM] in the presence of GOX [0.1 mg mL^{-1}] in PBS (pH 8.3) at 25 °C, upon addition of the 0.1 equivalents of H_2O_2. The concentrations of the dendrons that gave identical glucose release are indicated.

Conclusions

In summary, we have demonstrated a new 2CDCR probe system for detection of hydrogen peroxide, which exponentially amplifies a visual diagnostic signal. The system is composed of a simple chromogenic probe and a self-immolative dendron component that acts as a molecular amplifier. Four dendrons

with various numbers of glucose end-units were evaluated for the ability to produce exponential signal amplification through their self-immolative disassembly pathway. The release of free glucose molecules and their oxidation by GOX to produce hydrogen peroxide generated a chain reaction, which amplified a diagnostic signal. The self-immolative dendron based on an AB_3 platform exhibited the best characteristics of a probe system with rapid disassembly rate and good stability under aqueous conditions. The modularity and flexibility of a two-component detection system should allow extension for detection of other analytes.

Acknowledgements

D.S. thanks the Israel Science Foundation (ISF), the Binational Science Foundation (BSF) and the German–Israeli Foundation (GIF) for financial support.

References

1 M. S. Baker and S. T. Phillips, *J. Am. Chem. Soc.*, 2011, **133**, 5170–5173.
2 J. M. Nam, C. S. Thaxton and C. A. Mirkin, *Science*, 2003, **301**, 1884–1886.
3 A. Sagi, R. Weinstain, N. Karton and D. Shabat, *J. Am. Chem. Soc.*, 2008, **130**, 5434–5435.
4 T. Sano, C. L. Smith and C. R. Cantor, *Science*, 1992, **258**, 120–122.
5 (*a*) E. Sella and D. Shabat, *Chem. Commun.*, 2008, 5701–5703; (*b*) L.-C. Lo and C.-Y. Chu, *Chem. Commun.*, 2003, 2728–2729.
6 S. W. Thomas 3rd, G. D. Joly and T. M. Swager, *Chem. Rev.*, 2007, **107**, 1339–1386.
7 R. Weinstain, A. Sagi, N. Karton and D. Shabat, *Chem.–Eur. J.*, 2008, **14**, 6857–6861.
8 Q. Wu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 14682–14683.
9 H. J. Yoon and C. A. Mirkin, *J. Am. Chem. Soc.*, 2008, **130**, 11590–11591.
10 L. Zhu and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 2006, **45**, 1190–1196.
11 M. Avital-Shmilovici and D. Shabat, *Bioorg. Med. Chem.*, 2010, **18**, 3643–3647.
12 E. Sella and D. Shabat, *J. Am. Chem. Soc.*, 2009, **131**, 9934–9936.
13 R. J. Amir, N. Pessah, M. Shamis and D. Shabat, *Angew. Chem., Int. Ed.*, 2003, **42**, 4494–4499.
14 K. Haba, M. Popkov, M. Shamis, R. A. Lerner, C. F. Barbas 3rd and D. Shabat, *Angew. Chem., Int. Ed.*, 2005, **44**, 716–720.
15 A. Sagi, E. Segal, R. Satchi-Fainaro and D. Shabat, *Bioorg. Med. Chem.*, 2007, **15**, 3720–3727.
16 E. Sella, A. Lubelski, J. Klafter and D. Shabat, *J. Am. Chem. Soc.*, 2010, **132**, 3945–3952.
17 E. Sella, R. Weinstain, R. Erez, N. Z. Burns, P. S. Baran and D. Shabat, *Chem. Commun.*, 2010, **46**, 6575–6577.
18 R. Cheaib, A. Listkowski, S. Chambert, A. Doutheau and Y. Queneau, *Tetrahedron: Asymmetry*, 2008, **19**, 1919–1933.