Intramolecular base-accelerated radical-scavenging reaction of a planar catechin derivative bearing a lysine moiety†

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A planar catechin derivative incorporating a lysine moiety was synthesized and showed ~400-fold increased radical-scavenging activity relative to naturally-occurring (+)-catechin.

Oxidative stress contributes to the incidence of brain dysfunction, cancer, cardiovascular diseases, and inflammation.[1–4] The prevention of such diseases by using phenolic antioxidants such as flavonoids and vitamin E is receiving increasing attention.[5–8] The radical-scavenging activity of phenolic antioxidants, essential for prevention of such oxidative stress related diseases, is based on their redox properties which are dominated by the rate of hydrogen transfer reactions between the phenolic antioxidant and reactive oxygen species.[9] Among the different antioxidants, particular attention has focused on (+)-catechin (**1**) which naturally occurs in green tea.[10–12] The structural feature responsible for the radical-scavenging activity of (+)-catechin is the *ortho*-dihydroxy functionality in the catechol ring.[13–15] Introduction of electron-donating or withdrawing substituents into this catechol ring might regulate the radical-scavenging activities by altering the redox property of catechin. In addition, other chemical modifications affecting O–H bond dissociation enthalpy or stabilization of the phenoxyl radical might also be effective for improving antioxidative activities. In this context, we previously synthesized a planar catechin analogue (**2**), in which the catechol ring and chromane structure in **1** are constrained to be planar. This analogue showed an approximate 5-fold increase in radical-scavenging activity compared with **1**.[16,17] The predominant radical-scavenging activity of **2** is attributed to a more negative oxidation potential than **1**.[18] We also synthesized planar catechin derivatives having various alkyl side chain lengths.[19] It was found that increasing the number of carbon atoms included in the alkyl chain resulted in increasing radical scavenging abilities and oxidative protection.

Herein, we describe a new type of planar catechin derivative with a lysine moiety as a basic substituent with the aim to improve antioxidant and radical-scavenging activities. This idea is based on our previous report that the radical-scavenging reaction of a vitamin E model is significantly accelerated by the presence of a base, such as pyridine.[20] As vitamin E is oxidized to form the corresponding radical cation by a radical-scavenging reaction, the acceleration is attributed to the stabilization of the radical cation by base. Therefore, if a basic functional group were introduced into **2**, the radical-scavenging activities of **2** would be increased even in neutral conditions without addition of base, thereby leading to the development of a super antioxidant effective for the prevention and treatment of oxidative stress-related diseases.

In the design of the planar catechin derivative, the orientation of the amino substituent with respect to the catechol structure of planar catechin was especially taken into account. The amino substituent should have a similar conformation to the catechol structure in **2** so as to stabilize the radical cation of catechol formed by reaction with various types of reactive oxygen species. The planar catechin derivative (**3**) designed in this study is shown in Fig. 1. The lysine moiety in **3** is incorporated into the planar catechin through a hydroxy propyl group as an appropriate linker that defines a precise geometry between the planar catechin and lysine. The conformational space of **3** was explored by DFT (B3LYP/6-31G*) calculations. As shown in Fig. 2, the design provides a favorable relationship between the amino functional group and the hydroxyl group of the catechol structure. The distance between the 14-OH group of the catechol and N of the amino group is 1.78 Å, indicating that formation of an intramolecular O–H···N hydrogen bond is conformationally favorable which would enhance radical-scavenging activities. In addition, the ionization potential of **3**, calculated by DFT, is 6.14 eV which is much lower than that of **2** (7.38 eV), suggesting a higher potential for the one-electron oxidation reaction responsible for radical-scavenging activities.

The overall strategy for the synthesis of **3** is outlined in Fig. 3. Treatment of **1** with 4-oxovaleric acid ethyl ester resulted in the formation of a planar catechin structure with an alkyl substituent (**4**). After protection of phenolic OH with benzyl bromide, the ethyl ester of **4** was reduced with LAH to

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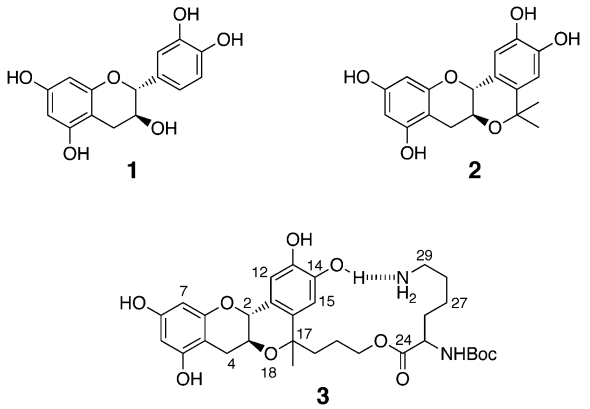

Fig. 1 Structures of **1**, **2** and **3**.

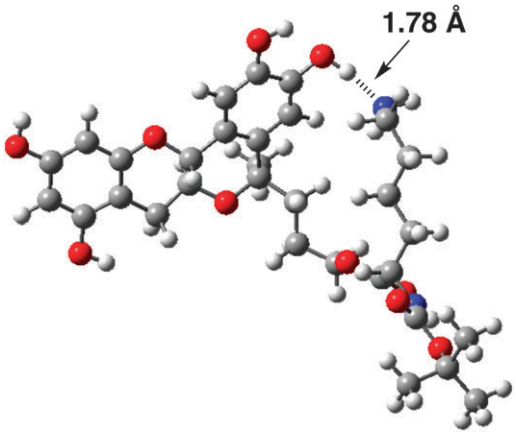

Fig. 2 DFT (density functional theory) optimized structure of **3** calculated using the B3LYP/6-31G* basis set.

afford the alcohol derivative (**5**), which was then reacted with N^{α}-Boc-N^{ε}-Z-Lys using DCC, followed by deprotection with hydrogenation (Pd/C, H_2) to furnish **3**. In the NOESY spectrum, cross-peaks were observed for H15/H27 and H15/H29 (data not shown), revealing that the amino group was spatially close to the catechol structure and formed interactions *via* hydrogen bonding. The radical-scavenging properties of **3** were tested in a non-aqueous system using the galvinoxyl radical (GO•) as an oxyl radical species. When **3** was added to a deaerated acetonitrile solution of GO•, the visible absorption band at 428 nm due to GO• disappeared immediately, indicating that the GO•-scavenging reaction of **3** was taking place. As the spectrum change is very fast, the rate of the radical-scavenging reaction of **3** was measured by monitoring the decrease in absorbance at 428 nm due to GO• using the stopped-flow technique. The decay of the absorbance of 428 nm obeyed pseudo-first-order kinetics when the concentration of **3** was maintained at a more than 10-fold excess of the GO• concentration. The pseudo-first-order rate constant (k_{obs}) increased linearly with an increase in concentration of **3** as shown in Fig. 4. From the slope of the linear plot of k_{obs} *vs.* [**3**], the second-order rate constant (k) for the radical-scavenging reaction was determined as 1.1×10^4 M^{-1} s^{-1}. The k values for **1** and **2** were determined in the same manner to be 2.6×10 and 1.5×10^2 M^{-1} s^{-1}, respectively. These results indicated

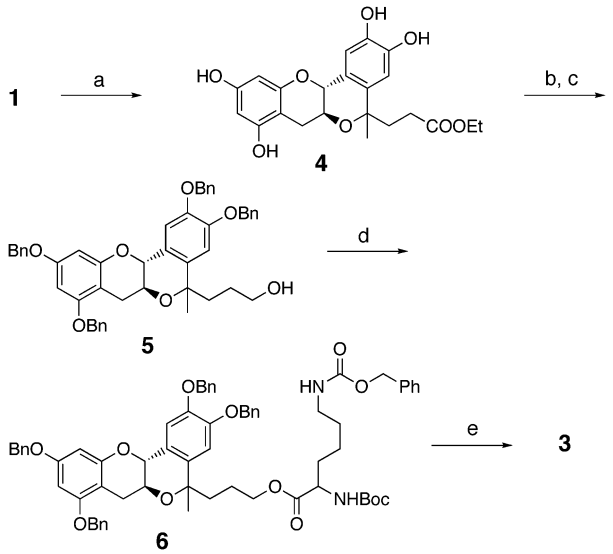

Fig. 3 Synthesis of **3**: (a) ethyl 4-oxovalerate, TMSOTf, THF (76%); (b) benzyl bromide, K_2CO_3, DMF (90%); (c) $LiAlH_4$, THF (92%); (d) N^{α}-Boc-N^{ε}-Z-Lys, DCC, DMAP, CH_2Cl_2 (62%); (e) H_2, 10% Pd/C, THF (81%).

that the introduction of the lysine moiety increases the k value compared to **2** by 73 times, or a 420-fold increase over the k value of **1**. The k value of **3** is remarkably high and to our knowledge is indeed the strongest antioxidant ever observed.

There are two possibilities for the mechanism of the radical-scavenging reaction toward the oxyl radical: either a one-step hydrogen atom transfer or an electron transfer followed by a proton transfer.[20,21] Previously, **1** and **2** were found to scavenge the oxyl radical by an electron transfer mechanism *via* the formation of a radical cation intermediate.[18] In analogy with planar catechin **2**, the GO•-scavenging reaction of **3** is thought to proceed by a mechanism involving an electron transfer from **3** to GO• rather than a one-step hydrogen atom transfer. Therefore, the amino group in lysine, which is located close to the catechol moiety and participates in intramolecular hydrogen bonding, is capable of stabilizing the radical cation intermediate $\mathbf{3}^{\bullet+}$ generated in the electron-transfer oxidation of **3** by GO•, resulting in the enhanced radical-scavenging activity. In

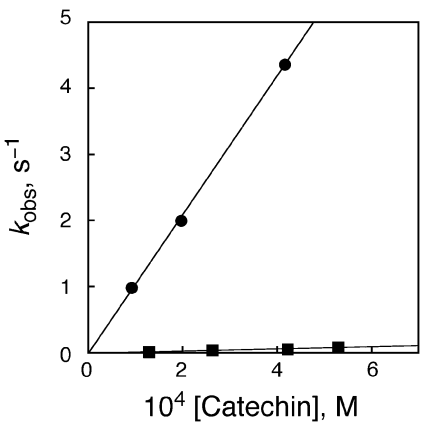

Fig. 4 Plot of the pseudo-first-order rate constants (k_{obs}) *vs.* [catechin] for the reaction of **2** (■) or **3** (●) with GO• (2.4×10^{-5} M) in deaerated MeCN at 298 K.

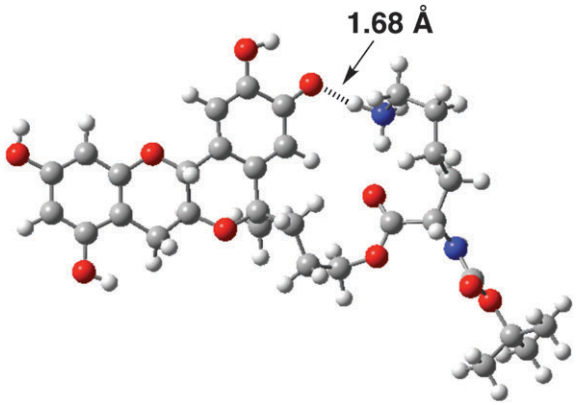

Fig. 5 DFT optimized structure of $\mathbf{3}^{\bullet+}$ calculated using the B3LYP/6-31G* basis set.

contrast, if the radical-scavenging mechanism proceeds *via* the formation of **3(-H)•** by a one-step hydrogen atom transfer mechanism, no interaction between the amino group and **3(-H)•** will occur. The structure of $\mathbf{3}^{\bullet+}$ was also optimized using DFT calculations. As shown in Fig. 5, the optimized geometry of $\mathbf{3}^{\bullet+}$ indicated that the proton of the 14-OH group had been transferred to the amino group of the lysine and the length of the resultant O···H–N hydrogen bond was 1.68 Å. This intramolecular proton transfer displacing hydrogen bonding into O···H–N significantly contributes to the stabilization of $\mathbf{3}^{\bullet+}$, resulting in an enhancement in the radical-scavenging activity of **3** by the electron transfer mechanism as depicted in Fig. 6.

In conclusion, a planar catechin derivative incorporating a lysine moiety was constructed, based on the concept that the radical cation generated from the reaction between **3** and an oxyl radical is stabilized by the base functionality of the lysine side-chain, thereby enhancing radical-scavenging activities. By observing the reaction with GO•, **3** showed significantly

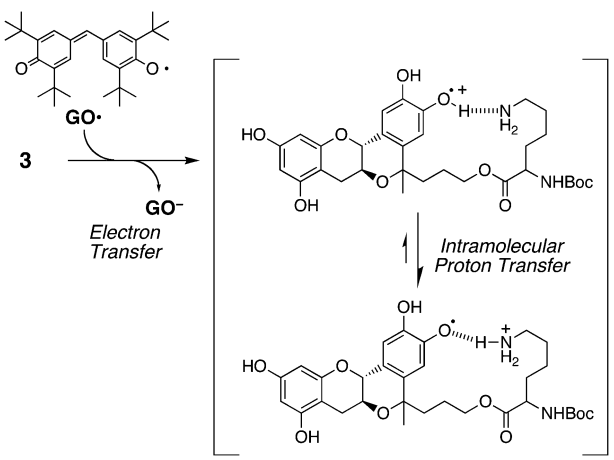

Fig. 6 Proposed radical-scavenging mechanism of **3**.

stronger radical-scavenging activity compared to **2**. The intramolecular hydrogen bond O–H···N between the amino moiety in lysine and 14-OH in catechol was validated by a DFT calculation of **3**. Proton transfer from 14-OH to the amino group, thereby displacing the hydrogen bonding to O···H–N, was also shown in the optimized geometry of $\mathbf{3}^{\bullet+}$. Stabilization of the radical cation arising from this hydrogen bond by the amino group of lysine results in the enhancement of radical-scavenging activity. This study improves our understanding of the radical-scavenging mechanism of phenolic antioxidants, and contributes to the further development of synthetic antioxidants with strong radical-scavenging activities.

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