アントラキノン-ヒドラジドハイブリッドによる還元糖の選択的光分解とAGEs生成阻害への応用

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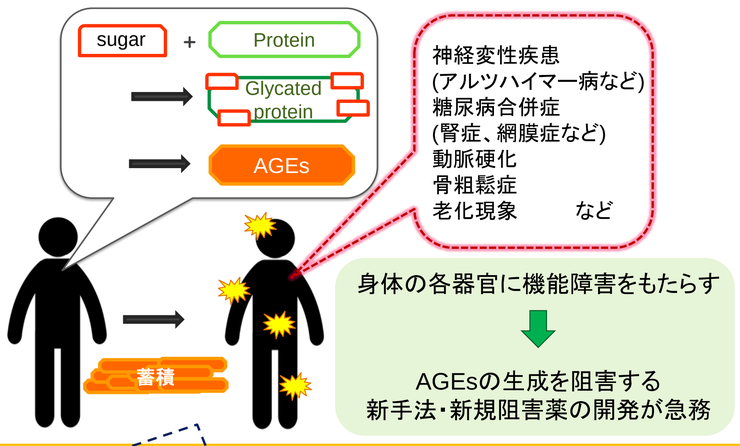
1. Introduction

1.1 タンパク糖化反応

生体内においてタンパクが糖と結びつくことで、
変性、劣化し、AGEs(糖化最終生成物)が生成する反応

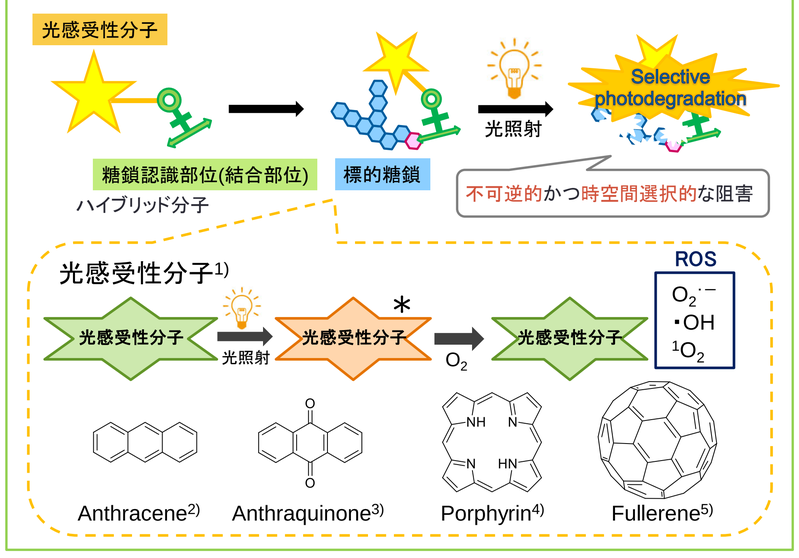


神経変性疾患
(アルツハイマー病など)
糖尿病合併症
(腎症、網膜症など)
動脈硬化
骨粗鬆症
老化現象　　など

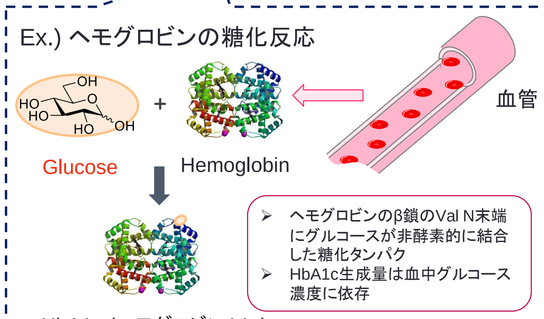
身体の各器官に機能障害をもたらす

AGEsの生成を阻害する
新手法・新規阻害薬の開発が急務

1.3 糖の光分解

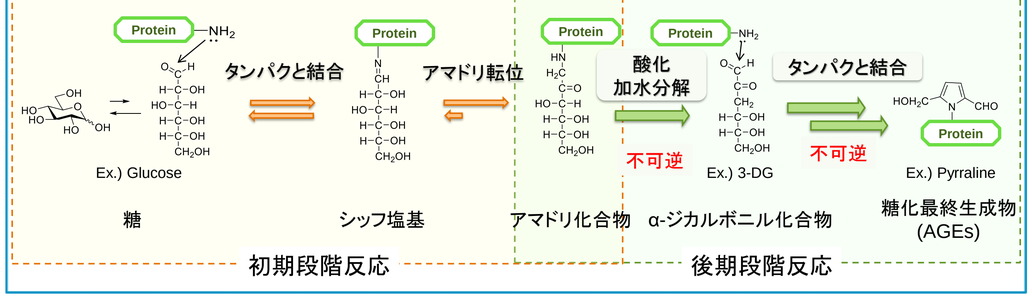


Ex.) ヘモグロビンの糖化反応



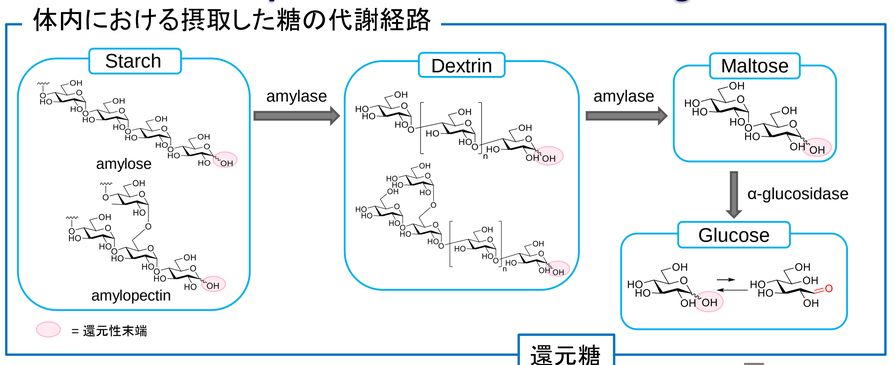
- ヘモグロビンのβ鎖のVal N末端にグルコースが非酵素的に結合した糖化タンパク
- HbA1c生成量は血中グルコース濃度に依存

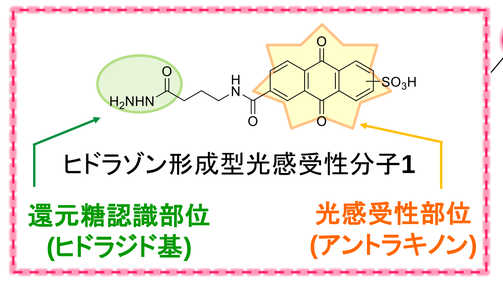
1.2 糖化反応プロセス

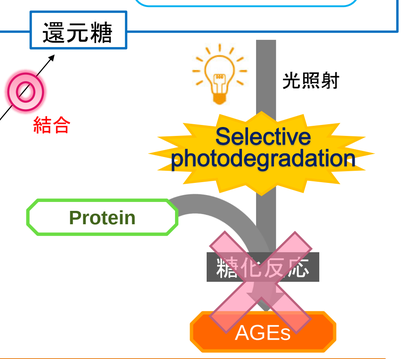


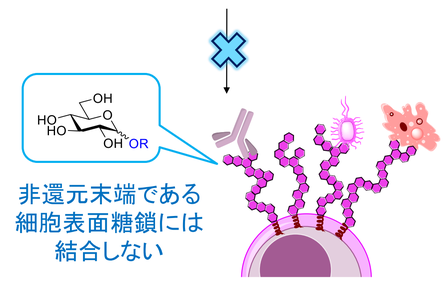
2. Research Purpose and Molecular Design

体内における摂取した糖の代謝経路







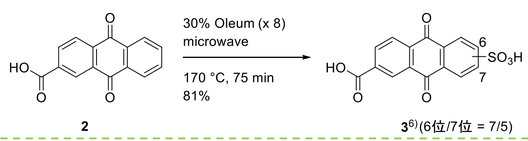


還元糖の選択的光分解による
糖化反応の制御

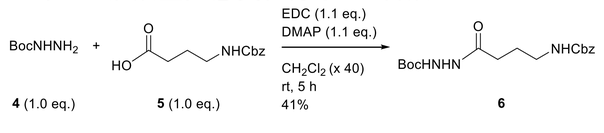
AGEsの生成を抑制

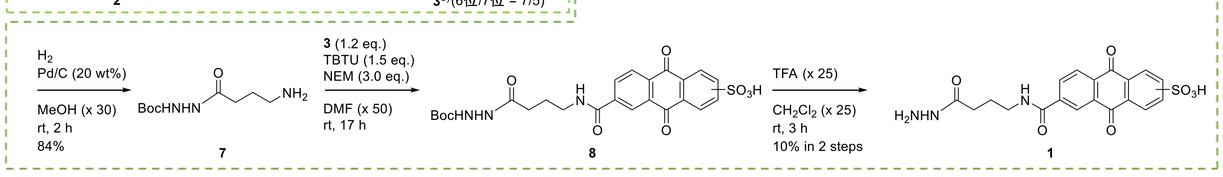
3. Synthesis of Hybrid 1

水溶性アントラキノン3の合成

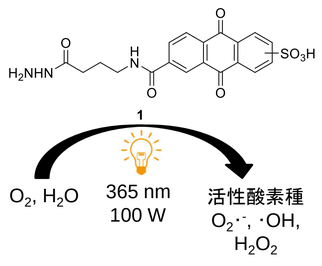


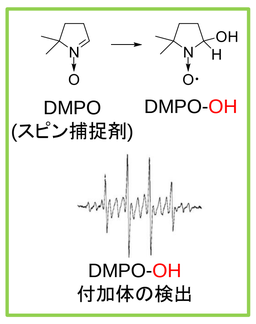
ヒドラゾン形成型光感受性分子1の合成





4. ESR Analysis





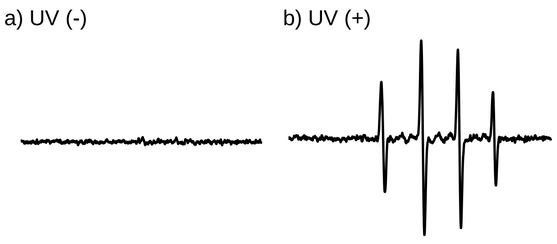
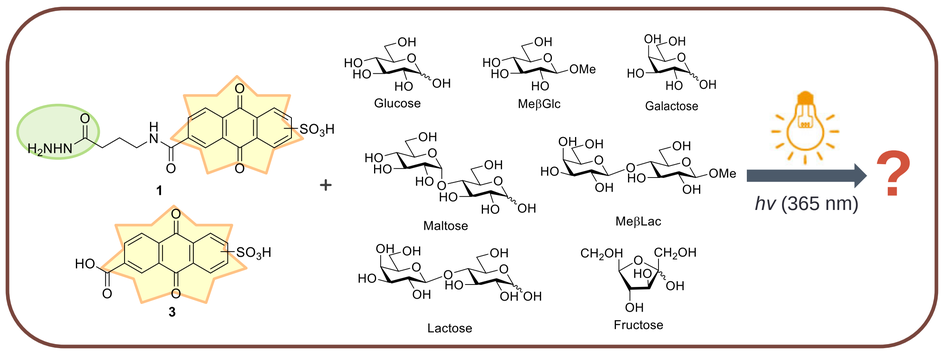
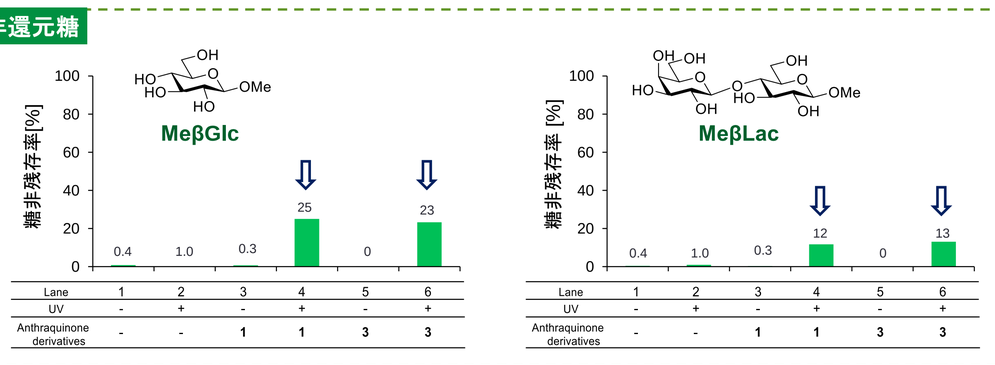


Figure 1 ESR spectra obtained during photoirradiation of **1** in the presence of DMPO. **1** (500 μM) and DMPO (500 mM) were incubated in 2% DMSO/H_2O containing 1 mM DETAPAC under irradiation with a UV lamp (365 nm, 100 W) placed 40 cm from a flat cell. a) Before irradiation. b) After 10 min irradiation. DETAPAC=diethylenetriaminepentaacetic acid.

5. Selective Photodegradation Assay for Reducing Sugars



非還元糖

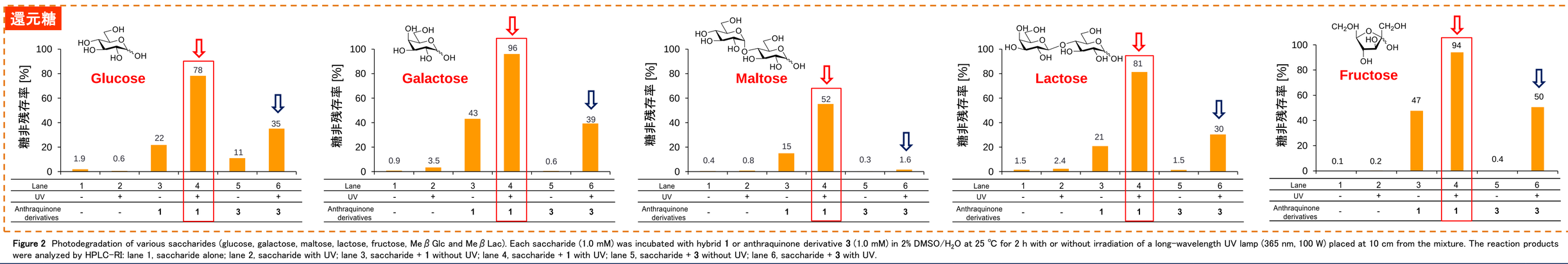


Lane	1	2	3	4	5	6
UV	-	+	-	+	-	+
Anthraquinone derivatives	-	-	**1**	**1**	**3**	**3**

1は非還元糖に対して光照射下、
3と同程度の光分解活性を発現

1は還元糖に対して光照射下、選択的
かつ効果的な光分解活性を発現

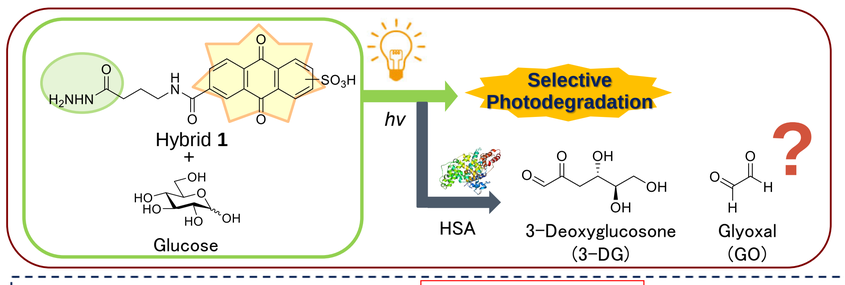
還元糖

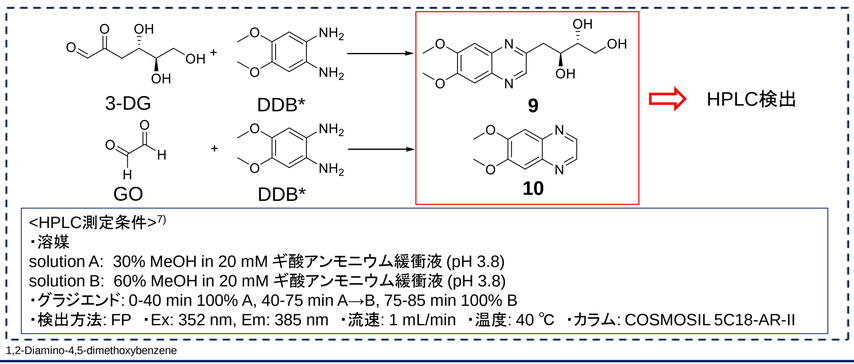


Lane	1	2	3	4	5	6
UV	-	+	-	+	-	+
Anthraquinone derivatives	-	-	**1**	**1**	**3**	**3**

Figure 2 Photodegradation of various saccharides (glucose, galactose, maltose, lactose, fructose, Meβ Glc and Meβ Lac). Each saccharide (1.0 mM) was incubated with hybrid **1** or anthraquinone derivative **3** (1.0 mM) in 2% DMSO/H_2O at 25 ℃ for 2 h with or without irradiation of a long-wavelength UV lamp (365 nm, 100 W) placed at 10 cm from the mixture. The reaction products were analyzed by HPLC-RI: lane 1, saccharide alone; lane 2, saccharide with UV; lane 3, saccharide + **1** without UV; lane 4, saccharide + **1** with UV; lane 5, saccharide + **3** without UV; lane 6, saccharide + **3** with UV.

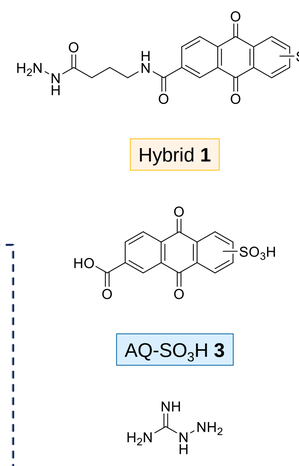
6. Inhibition of AGE-precursors Formation Using Hybrid 1 and AQ Derivative 3 with Photo-Irradiation

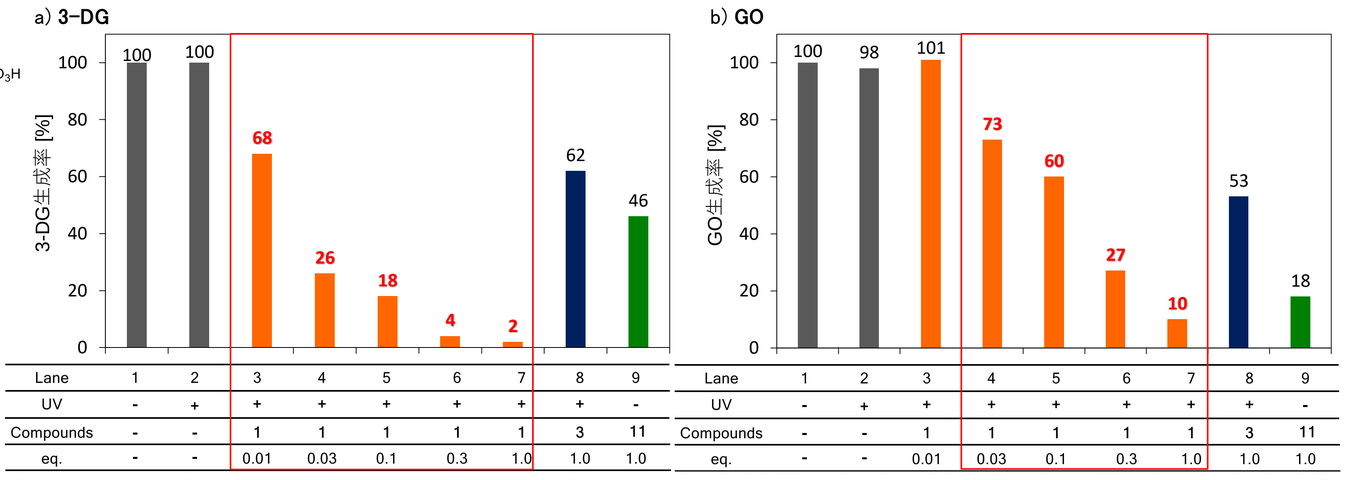




<HPLC測定条件>[7]
・溶媒
solution A: 30% MeOH in 20 mM ギ酸アンモニウム緩衝液 (pH 3.8)
solution B: 60% MeOH in 20 mM ギ酸アンモニウム緩衝液 (pH 3.8)
・グラジエント: 0-40 min 100% A, 40-75 min A→B, 75-85 min 100% B
・検出方法: FP　・Ex: 352 nm, Em: 385 nm　・流速: 1 mL/min　・温度: 40 ℃　・カラム: COSMOSIL 5C18-AR-II

* 1,2-Diamino-4,5-dimethoxybenzene





Lane	1	2	3	4	5	6	7	8	9
UV	-	+	+	+	+	+	+	+	-
Compounds	-	-	1	1	1	1	1	3	11
eq.	-	-	0.01	0.03	0.1	0.3	1.0	1.0	1.0

Figure 3 Inhibition of AGE-precursors ((a) 3-DG and (b) GO) Formation. Glucose (50 mM) was incubated with hybrid **1**, anthraquinone derivative **3** or aminoguanidine **11** in sodium phosphate buffer (pH 7.4, 100 mM) at 25 ℃ for 2 h with or without irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the mixture, and then HSA (50 mg/mL) was added into the mixture. After incubation at 60 ℃ for 30 h, the reaction mixture was quenched by addition of ice-cold perchloric acid (0.6 M) and kept on ice for 10 min. The samples containing HSA were centrifuged (10000 *g*, 5 min) at 4 ℃. The supernatant was transferred into glass vials and the derivatizing agent, DDB (1.11 mM), was added, and then samples were incubated at room temperature in the dark for 12 h. The pH of the samples were adjusted to 2.3 with Na_2HPO_4 (0.5 M). The samples were applied to solid phase extraction cartridge (ODS, 500 mg) equilibrated with 20 mM ammonium phosphate, pH 2.3. The quinoxaline analytes **9** and **10** eluted with methanol. The methanol samples were analyzed by HPLC-FP. Lane 1, control; lane 2, UV only; lane 3, **1** (0.01 eq.) with UV; lane 4, **1** (0.03 eq.) with UV; lane 5, **1** (0.1 eq.) with UV; lane 6, **1** (0.3 eq.) with UV; lane 7, **1** (1.0 eq.) with UV; lane 8, **3** (1.0 eq.) with UV; lane 9, **11** (1.0 eq.) without UV.

7. Conclusions

●ヒドラゾン形成型光感受性分子**1**の合成を達成した。
●ヒドラゾン形成型光感受性分子**1**の還元糖(glucose，galactose，maltose及びlactose)と非還元糖(MeβGlc及びMeβLac)に対する光分解活性をHPLCを用いて評価した結果、**1**が、人体に無害な長波長紫外光の照射下、ヒドラジド部位を有さないアントラキノン誘導体**3**と比べ、還元糖を効果的かつ選択的に光分解することを見出した。
●ヒドラゾン形成型光感受性分子**1**が光照射下、グルコースを選択的かつ効果的に分解することで、3-DG及びGO(AGEs前駆体)の生成を抑制する新たな生体機能光制御分子であることを見出した。

8. References

[1] Review: Toshima, K. *Mol. BioSyst.* **2013**, *9*, 834-854.
[2] Nishibu, M.; Takahashi, D.; Toshima, K. *Org. Biomol. Chem.* **2012**, *10*, 8393-8395.
[3] Aoki, Y.; Tanimoto, S.; Takahashi, D.; Toshima, K. *Chem. Commun.* **2013**, *49*, 1169-1171.
[4] Okochi, A.; Tanimoto, S.; Takahashi, D.; Toshima, K. *Chem. Commun.* **2013**, *49*, 6027-6029.
[5] Tanimoto, S.; Sakai, S.; Kudo, E.; Okada, S.; Matsumura, S.; Takahashi, D.; Toshima, K. *Chem. Asian J.* **2011**, *7*, 911-914.
[6] Imai, Y.; Hirono, S.; Matsuba, H.; Suzuki, T.; Kobayashi, Y.; Kawagishi, H.; Takahashi, D.; Toshima, K. *Chem. Asian J.* **2012**, *7*, 97-104.
[7] Thornalley, P. J.; Langborg, A.; Minhas, H. S. *Biochem. J.* **1999**, *344*, 109-116.