Communications to the Editor

Total Synthesis of the Ocular Age Pigment A2-E: A Convergent Pathway

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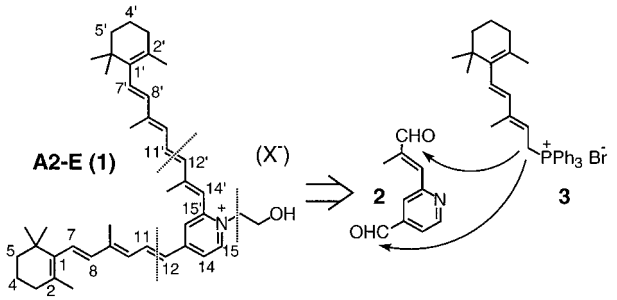
The autofluorescent age pigments (lipofuscin) which accumulate in the human retinal pigment epithelium (RPE) cells with age are considered to be responsible for causing various eye diseases including age-related macular degeneration (AMD), the leading cause of blindness in elderly people for which no remedy exists.[1] The prevalence associated with AMD among Americans above 40 years of age was estimated to be 9.2%,[2] and a rising tide in AMD is foreseen with the increase of median age.

The nature of lipofuscin accumulation in RPE remains a mystery. It is thought that the pigments are accumulative debris resulting from incomplete digestion of phagocytosized outer segment disks in lysosomes, which contain autofluorescent retinoids.[3] Among them, the orange fluorophores have attracted wide interest because of their suggested involvement in age-related decline in photoreceptor cell function. The structure originally assigned[4] to the major orange fluorophore isolated from over 250 donor eyes has been revised to **1** (Scheme 1) through a biomimetic reaction of 2 equiv of retinal (vitamin A) and 1 equiv of ethanolamine (thus the name "A2-E"; 0.5% yield after extensive chromatography) and structural studies.[5] The unprecedented wedge shape of this amphiphilic compound should be noted (Scheme 1).[6] Preliminary assays with human fibroblast lysosomes and red blood cells suggest that A2-E could be a factor leading to the lipofuscin formation.[7] Studies also suggest that A2-E induce phototoxicity on human RPE,[8] indicating that photodynamic inactivation of photoreceptor cells may lead to AMD.

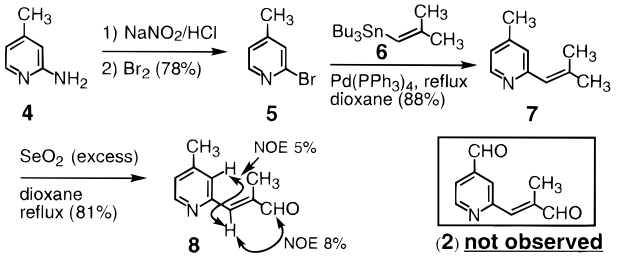
We report herein the total synthesis of A2-E via a convergent double Wittig olefination of bis-aldehyde **2** with Wittig reagent **3**[9] containing the moiety common to both side arms (Scheme 1), followed by alkylation at the pyridine nitrogen. The initial attempted route to the key bis-aldehyde **2** is illustrated in Scheme 2. Thus, 2-bromo-4-methylpyridine (**5**), prepared from 2-amino-4-methylpyridine (**4**) by diazotization and bromination[10] was subjected to Stille coupling reaction[11] with tin reagent **6** (prepared by lithiation of 1-bromo-2-methyl-1-propene followed by treatment with tributylstannyl chloride[12]) to give **7**. Oxidation of **7** with SeO_2 under various conditions gave, instead of the desired bis-aldehyde **2**, mono-aldehyde **8** as the sole product with oxidation occurring on the less-hindered methyl group (Scheme 2).[13] The inability of the 4-methyl group to undergo further oxidation to **2** is presumably due to the electron-withdrawing nature of the enal moiety in **8** and coordination of SeO_2 to the pyridine nitrogen. In support of this coordination, addition of SeO_2 to **7** or **8** in $CDCl_3$ induced substantial downfield shifts of all proton NMR signals by 0.8−1.2 ppm. When 2-bromo-4-methylpyridine (**5**) was subjected to SeO_2 oxidation, no trace of 2-bromo-4-formylpyridine was formed, presumably due to the inductive effect of the Br substituent.

We then chose 4-methylpyridone (**9**) as the starting material.[14] The NMR olefinic proton signals (in $CDCl_3$) of **9** appear at 6.5−7.2 ppm, showing that it exists exclusively in the pyridone form.[15] Addition of SeO_2, however, results in downfield shifts of >1.2 ppm, indicating that **9** tautomerizes to 2-hydroxy-4-methylpyridine (**10**) by coordination of SeO_2 to the nitrogen atom with concomitant hydrogen-bond formation, as in **11**.

Scheme 1



Scheme 2



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(1) (a) *Vision Problems in the United States: Data Analysis;* National Society for the Prevention of Blindness: New York, 1980; pp 1−46. (b) Hyman, L. In *Age-Related Macular Degeneration: Principles and Practice*; Hampton, G. R., Nelson, P. T., Eds.; Raven Press: New York, 1992; pp 1−35. (c) Evans, J. R.; Wormald, R. P. L. *Invest. Opthalmol. Visual Sci.* **1994**, *35*, 2003.

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(3) Eldred, G. E. in *Retinal Degeneration. Clinical and Laboratory Applications*; Hollyfield, J. G., Anderson, R. E., LaVail, M. M., Eds.; Plenum Press: New York, 1993; pp 15−24.

(4) (a) Eldred, G. E.; Lasky, M. R. *Nature (London)* **1993**, *361*, 724. (b) Eldred, G. E. *Nature (London)* **1993**, *364*, 396.

(5) Sakai, N.; Decatur, J.; Nakanishi, K.; Eldred, G. E. *J. Am. Chem. Soc.* **1996**, *118*, 1559. The numbering of carbon in structure **1** (Scheme 1) refers to those in the retinoid side chains, thus indicating its biogenesis from the retinal (vitamin A).

(6) Fuhrhop, J.-H.; Koning, J. *Membranes and Molecular Assemblies: The Synkinetic Approach*; The Royal Society of Chemistry: Cambridge, 1994; Chapter 3, p 28.

(7) (a) Eldred, G. E. *Gerontology* **1995**, *41*, 15. (b) Eldred, G. E.; Katz, M. L. *Exp. Eye Res.* **1988**, *47*, 71.

(8) Organisciak, D. T.; Winkler, B. S. *Prog. Retinal Eye Res.* **1994**, *13*, 1.

(9) (a) Loeber, D. E.; Russell, S. W.; Toube, T. P.; Weedon, B. C. L.; Diment, J. *J. Chem. Soc. (C)* **1971**, 404. (b) Mayer, H. *Pure. Appl. Chem.* **1979**, 535.

(10) Allen, C. F. H.; Thirtle, J. R. *Organic Syntheses;* Wiley, New York, 1955; Collect. Vol. III, p 136.

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(13) SeO_2 undergoes ene-reaction at allylic and arylic positions by coordination to π-systems yielding allylic alcohols which are then oxidized to aldehydes. See: Bulman, P. C.; McCarthy, T. J. In *Comprehensive Organic Synthesis;* Trost, B. M., Fleming, I., Eds.; Pergamon Press: New York, 1993; Vol. 7, p 83.

(14) The methyl groups of 1,4-dimethylcarbostyril,[14a] 6-methyluracil,[14b] and triacetic lactone methyl ether[14c] undergo facile SeO_2 oxidation to aldehydes: (a) Cook, D. J.; Stamper, M. *J. Am. Chem. Soc.* **1947**, *69*, 1467. (b) Zee-Cheng, K. Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1967**, 163. (c) Suzuki, E.; Hamajima, R.; Inoue, S. *Synthesis* **1975**, 192.

(15) Boulton, A. J.; McKillop, A. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon Press: New York, 1993; p 56.

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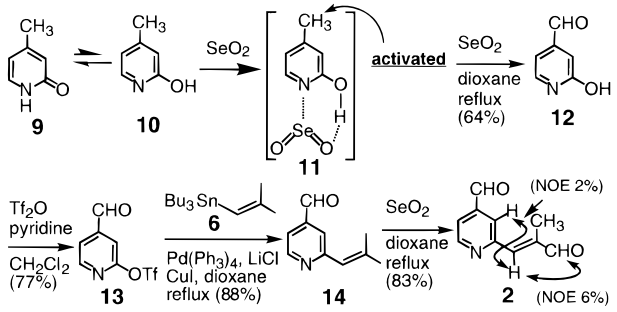
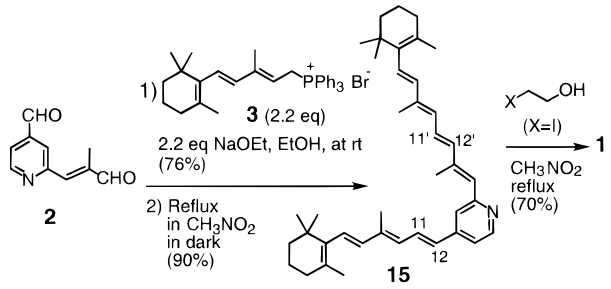
Scheme 3



Despite this coordination, the electron-donating nature of the OH group must be sufficient to result in oxidation of the methyl group to aldehyde. Thus, reaction of **9** with 1.2 equiv of SeO_2 gave 2-hydroxy-4-formylpyridine (**12**) in moderate yield (64%), which was converted to triflate **13** with Tf_2O/pyridine. Stille coupling with the tin reagent **6** in the presence of a catalytic amount of CuI afforded **14** (78%).[16] Oxidation of **14** with SeO_2 was both chemo- and regiospecific, providing bis-aldehyde **2** (83%) despite the presence of the electron-withdrawing 4-formyl group (Scheme 3).

Double Wittig olefination of bis-aldehyde **2** with 2.2 equiv of **3** afforded a mixture of four isomers at 11 and 11′ in 4:3:3:2 ratio. Attempts to isomerize this mixture to all-trans isomer using a catalytic amount of iodine[17] and to separate the isomers on silica gel column were both unsuccessful. However, when this 4:3:3:2 mixture was heated in nitromethane (bp 100 °C) under reflux overnight in the dark, the all-trans isomer **15** was observed as the predominant product (>90% conversion to **15**; stereochemistry determined by NMR), which was purified by preparative TLC (Scheme 4). Thus, the polyene chains with cis C═C bonds undergo thermally-induced isomerization.[17,18] Alkylation of all-trans **15** with iodoethanol in nitromethane

Scheme 4



(upon reflux overnight in the dark) followed by silica gel column purification yielded **1** (70%). The physical properties, i.e., ^1H and ^{13}C NMR, UV, IR, and MS, of this fluorescent isomer were identical to native A2-E.

This convergent synthesis of ocular age pigment A2-E **1** can be modified to include preparations of certain isotopically substituted analogs. A2-E and analogs will be used for critical mechanistic studies such as the mode of interactions with RPE cells, RPE enzymes, phototoxicity, and the metabolic changes of the A2-E molecule itself. If it can be proven that A2-E is indeed the main cause leading to AMD, the A2-E molecule can be used to seek for remedies, either by destroying the formed molecule in RPE cells and/or preventing its formation. An interdisciplinary approach will be taken for these studies.

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Supporting Information Available: Experimental procedures and spectral data (17 pages). See any current masthead page for ordering and Internet access instructions.

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(17) Sonnet, P. E. *Tetrahedron* **1980**, 557 and references cited therein.

(18) Alkylation of the 4:3:3:2 mixture with 2-iodoethanol under reflux in nitromethane in the dark also led to the formation of one predominant isomer which was identical to native A2-E. In another case, an isomeric mixture of cis/trans merocyanines was converted into the all-trans species upon formation of cyanine dye. See: Derguini, F.; Caldwell, C. G.; Motto, M. G.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 646.