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Total synthesis of AsLn2 – a luciferin analogue from the Siberian bioluminescent earthworm *Fridericia heliota*

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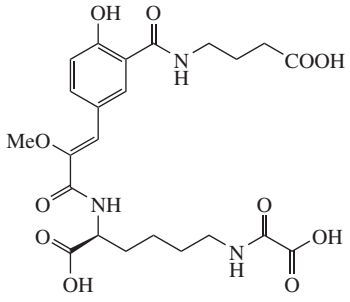
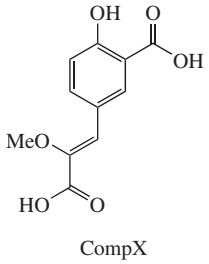
Total synthesis of AsLn2, a luciferin analogue isolated from the Siberian bioluminescent earthworm *F. heliota*, was performed from (*Z*)-5-(2,3-dimethoxy-3-oxoprop-1-en-1-yl)-2-hydroxybenzoic acid in six steps.

Bioluminescence – emission of 'cold light' by live species – generally results from oxidation of an organic compound termed luciferin promoted by a specific enzyme luciferase.[1] Until recently, only seven natural luciferins were known. In 2014 we reported structure elucidation and synthesis of a novel luciferin from the Siberian bioluminescent earthworm *Fridericia heliota* (Annelida: Clitellata: Oligochaeta: Enchytraeidae).[2] This luciferin turned out to be a nonribosomal modified peptide formed by the residues of oxalic acid, L-lysine, modified tyrosine and γ-aminobutyric acid.

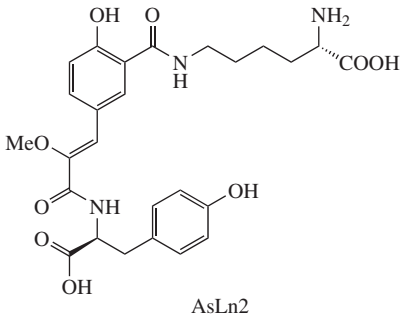
In contrast to a known bioluminescence mechanism of other worms, in which hydrogen peroxide is consumed to oxidize *N*-isovaleryl-3-aminopropanal in an ATP-independent manner,[3] *Fridericia*'s bioluminescent system was found to utilize ATP, Mg^{2+} ions, atmospheric oxygen and a specific (although still not fully characterized) luciferase.[4,5] We found *Fridericia* luciferin to possess an unprecedented mechanism of action, where oxidative decarboxylation of lysine residue occurs outside of the light-emitting fragment.† In the course of isolation and purification of *Fridericia* luciferin we found two abundant components in the worm biomass that showed chromatographic and UV spectral properties similar to luciferin. Using spectroscopic methods, their structures were established as (*Z*)-5-(2-carboxy-2-methoxyvinyl)-2-hydroxybenzoic acid designated CompX[6,7] and its diamide with lysine and tyrosine designated as AsLn2.[8] However, relative and absolute stereochemistry of AsLn2, which contains two stereogenic centers, has not been reported. Here we present synthesis of AsLn2 in order to establish its relative stereochemistry and provide route to the future studies of its biological functions.



Fridericia luciferin



CompX

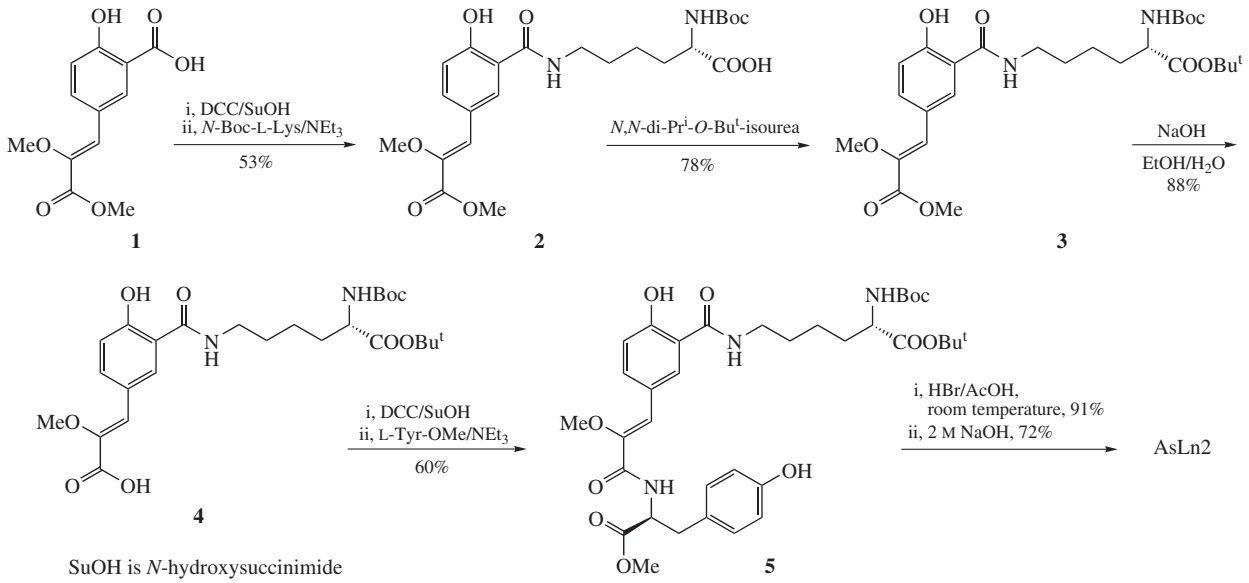


AsLn2

The synthesis of AsLn2 is outlined in Scheme 1.‡ CompX methyl ester **1**[7] was consecutively coupled with L-lysine and L-tyrosine using the standard non-racemization peptide chemistry. *N*-BOC and *O*-*tert*-butyl protective groups were used for lysine protection, which allowed one to perform simultaneous one-step cleavage using HBr in glacial acetic acid. The last step of AsLn2 synthesis was the base-catalyzed hydrolysis of methyl ester, used for the protection of tyrosine carboxyl.

The product of the last synthetic stage showed exact coincidence of its HPLC chromatographic and NMR characteristics (see Online Supplementary Materials) with those of the natural sample, implying the identity of the chemical connectivity and relative stereochemistry of the two molecules. The new NMR data obtained for synthetic AsLn2 forced us to reassign CompX carbons $C^{4'}$ (123.4 ppm) and $C^{6'}$ (117.6 ppm) in comparison to our previous publications.[7,8] The amount of the natural AsLn2 was not sufficient for determination of its optical rotation. However, the absolute stereochemistry of AsLn2 was deduced after chiral HPLC analysis of both natural and synthetic compounds, for which the retention times were practically identical. The obtained data indicated that both stereocenters of natural AsLn2 have L configuration and the structure for this compound was finally determined to be (*S*)-2-amino-6-(5-{(*Z*)-3-[(*S*)-1-

† The results will be published elsewhere.
‡ For procedures, see Online Supplementary Materials.



Scheme 1 Synthesis of AsLn2.

carboxy-2-(4-hydroxyphenyl)ethylamino]-2-methoxy-3-oxo-prop-1-en-1-yl}-2-hydroxybenzoylamino)hexanoic acid.

The exact biological function of AsLn2 is unclear. On the one hand, it may be a by-product in biosynthesis of *Fridericia* luciferin, resulting from erroneous coupling of CompX moiety to L-tyrosine and ε-amino group of L-lysine, instead of γ-amino-butyric acid and α-amino group of L-Lys. On the other hand, the presence of high amounts of CompX and its amides in the *Fridericia* biomass suggests that these compounds may play protective role. The structures of other luciferin analogues, their role in luciferin metabolism and their possible antibacterial and antifungal activities are presently under exploration in our group.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.03.005.

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