A novel synthesis of peptide-6-amino-D-luciferin conjugates for detection of peptidase activities

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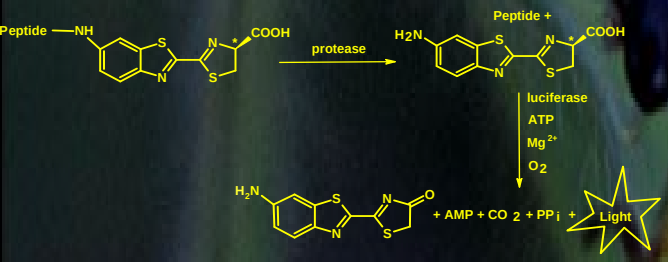
Biological application

In vivo bioluminescence imaging has become a cornerstone technology for preclinical molecular imaging. This imaging method is based on light-emitting enzymes, luciferases, which require specific substrates for light production. When linked to a specific biological process in an animal model of human biology or disease, the enzyme-substrate interactions become biological indicators that can be studied noninvasively in living animals [1].

Aminoluciferin *(aLuc)* is american firefly *(Photinus pyralis)* luciferin with its 6-position hydroxyl group substitued with an amino group. This modification allows *aLuc* to form amide bond with a peptide, while at the same time retaining the transport and bioluminescent properties of luciferin, resulting in a molecule called peptide-aminoluciferin. Many, particially protected peptide-aminoluciferin *(e.g. Z-Asp-Glu-Val-Asp-aLuc, Z-Leu-Glu-His-Asp-aLuc, Suc-Leu-Leu-Val-Tyr-aLuc)* are good substrates for bioluminescence assays, for example in the detection of protease actvity. Proteases represent important pharmaceutical targets because of their involvement in numerous disease processes [2]. The above mentioned conjugates generally offer significant advantages, such as increased sensitivity, ease of use, and high throughput screening capacity. Luciferase-based assays are typically 10- to 100-fold more sensitive than comparable fluorescent assays (Rhodamine 110, AMC and AFC) [3].

Light emission of the peptide-aminoluciferin



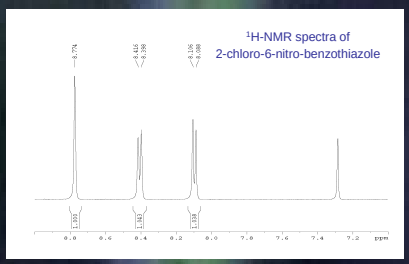
Synthetic problems

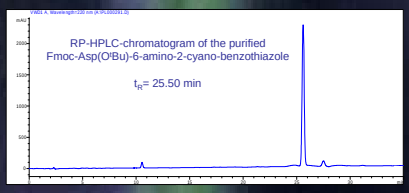
The synthesis of different type peptide-6-amino-D-luciferin conjugates and their precursors have been published [2] and some of them are commercially available. However, because of their high price the *in vivo* application of these conjugates is limited. To solve this problem we successfully worked out a new, easier and more convenient and economical method for the preparing these derivatives starting from 2-chloro-benzothiazole. Moreover this products have excellent purity (>99%) and adequate yield (82-93%).

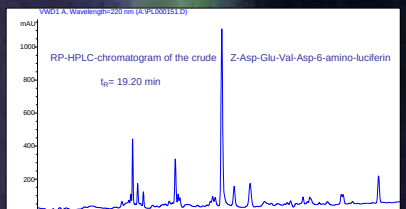
References

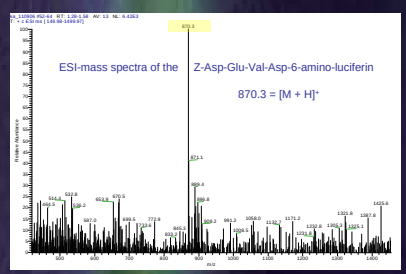
[1] *Biochemistry*, **2006**, *45*, 11103-11112.; [2] *J. of Biomol. Sc.*, **2005**, *10(2)*, 137-148.
[3] *BioTechniques*, **2011**, *51(2), 105-110.*

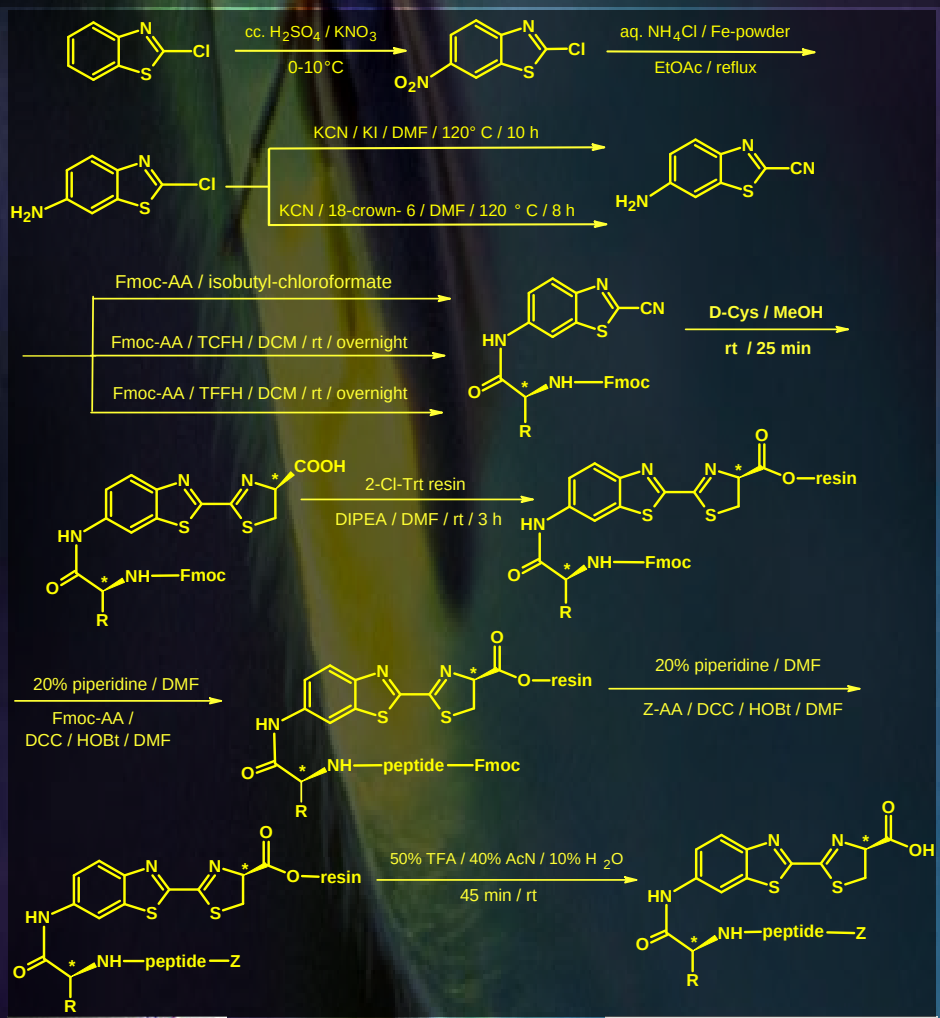
Synthesis



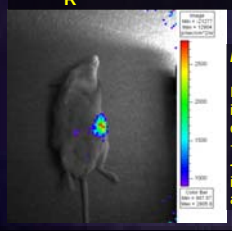








Biological tests

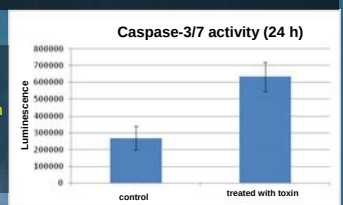


In vivo:

Immunosupressed SCID mice injected with A375luc melanoma cells + antitumour drug + Z-DEVD-aLuc
→ the measured luminescence indicate the increased caspase activity

In vitro:

Primary rat retinal cell culture + toxin (thapsigargin)
→ measurement of caspase activity



Acknowledgement

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