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Perspectives on the synthesis and use of ageladine A

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ABSTRACT

Focusing on the marine-derived alkaloid ageladine A ([4-(4,5-dibromo-1*H*-pyrrol-2-yl)]-1*H*-imidazo[4,5-*c*]pyridin-2-amine trifluoroacetate), we combined and modified published strategies to develop a synthesis method with easily managed reaction steps that allows gram-scale batch synthesis. On exploration additional features of the fluorescent properties of the compound were revealed. In tissues and cells of a marine flatworm, the emission profile shifted to longer wavelengths than in water. The fluorescence emission maximum shifted around 30–450 nm and the profile showed sufficient intensity at approximately 550 nm and above.

Introduction

Since the initial extraction of ageladine A ([4-(4,5-dibromo-1*H*-pyrrol-2-yl)]-1*H*-imidazo[4,5-*c*]pyridin-2-amine trifluoroacetate) [**1**, Fig. 1] in 2003[1] several scientific publications have provided a clearer picture of its properties. In terms of total synthesis, biological and physical properties of the natural compound have been revealed as a discovery of high potential: ageladine A has antiangionetic effects[1,2] and can be used as pH-indicator[3–5] or pH-sensitive imaging dye[6,7] that can be used in the proof of viability[8] of cells and tissues. As a brominated molecule of small molecular mass it is characterized by its ability to permeate membranes and low toxicity, and offers a promising core structure for related structures with comparable properties.[9]

The literature offers several strategies to synthesize ageladine A. On the one hand two electrocyclizations have been described: Meketa and Weinreb published a 6π-2-azatrieneelectro-cyclization and a 6π-azaelectrocyclization followed by a Suzuki–Miyaura coupling.[10,11] Additionally Mineno et al. describe a build-up of the ageladine A-bicycle starting from simple pyridine compounds.[12] On the other hand ageladine A can easily be synthesized via a Pictet–Spengler reaction product.[13–16]

Results and discussion

Practicable, concise synthesis

Comparing all the different published opportunities to synthesize ageladine A we strictly defined limitations: looking for a highly efficient, short synthesis that can be performed without specialized laboratory equipment and that allows variation of constituents. The methods provided by Meketa and Weinreb[10,11] were disregarded due to the high level of complexity in their synthetic strategy and their limited options in the variation of substituents.

In order to have an easy access to closely related structures, regarding ageladine A, we selected synthesis via Pictet–Spengler reaction: here, a substitution of educts directly results in derivative products.

The key aspect of the selected method to obtain ageladine A is the synthesis of the first Pictet–Spengler reaction educt: 2-amino-histamine or a derivative thereof. Laboratory limitations excluded the use of diazomethane-chemistry as applied when starting with β-alanine.[17]

The synthetic path offers two options to begin with: starting with the bromination of 4-hydroxy-2-butanon [**2**] and the joined reaction of the formed bromide [**3**] with phthalimide potassium salt[18,19] or the preferred one-step synthesis with the highly toxic methyl vinyl ketone [**5**] and phthalimide.[20] Both lead to 2-(3-oxobutyl)isoindoline-1,3-dione [**4**]. After bromination of the terminal methyl group[20–22] the phthalimide-protected bromomethyl-ketone [**6**] is coupled with acetyl-guanidine to form

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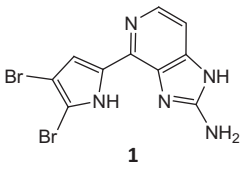


1

Figure 1. Chemical structure of ageladine A.

the imidazole ring [**7**].[23] The last step to yield 2-acetylamino-histamine is the deprotection of the terminal amino-group. This can be performed in different ways.[19,23] The procedure of Osby et al. in particular leads to a very clean reaction, depending on the pH during the adjusted work-up process.[24]

The Pictet–Spengler reaction uses 2-acetyl-aminohistamine [**8**] and 4,5-dibromo-1-SEM-pyrrol-2-carb-aldehyde [**9**] synthesized in a two-step synthesis starting with the bromination of pyrrol-2-carbaldehyde[25,26] and protection with trimethyl-silylethoxymethyl chloride (SEMCl).[15] This protecting group revealed itself as the most stable during the subsequent reaction steps and makes the chromatographic procedures easier, compared to the Boc-group. Without catalysis *N*′-SEM-5′-(2-acetylamino-4,5,6,7-tetrahydroimidazo[4,5-*c*]pyridin-4-yl)-2′,3′-dibromo-pyrrole [**10**] is yielded. The later dehydrogenation with activated manganese(IV) oxide leads to a doubly protected ageladine A [**11**].[27] This condition turned out to be applicable for the dehydrogenation of tetrahydropyridines or further condensed systems (e.g., tetrahydrocarbolines) and substitutes the oxidizing agents used to-date, for example, chloranil or IBX.[28] Finally, ageladine A [**1**] is available after cleavage of both protecting groups, performed in boiling ethanolic hydrochloric acid. The free base of ageladine A is precipitated as trifluoroacetate salt.

A large Stokes shift of ageladine A in living tissues

In living cells and tissues of the marine flatworm *Macrostomum lignano* we observed an increased Stokes shift when compared to the literature.[3] Different ionic strengths do not influence the exc./em. profiles (data not shown) of ageladine A but lipids and other organic molecules in combination with ionic strength may do so. The environment of a fluorophore strongly influences spectral properties in many ways as summarized and extensively discussed by Lakowicz.[29]

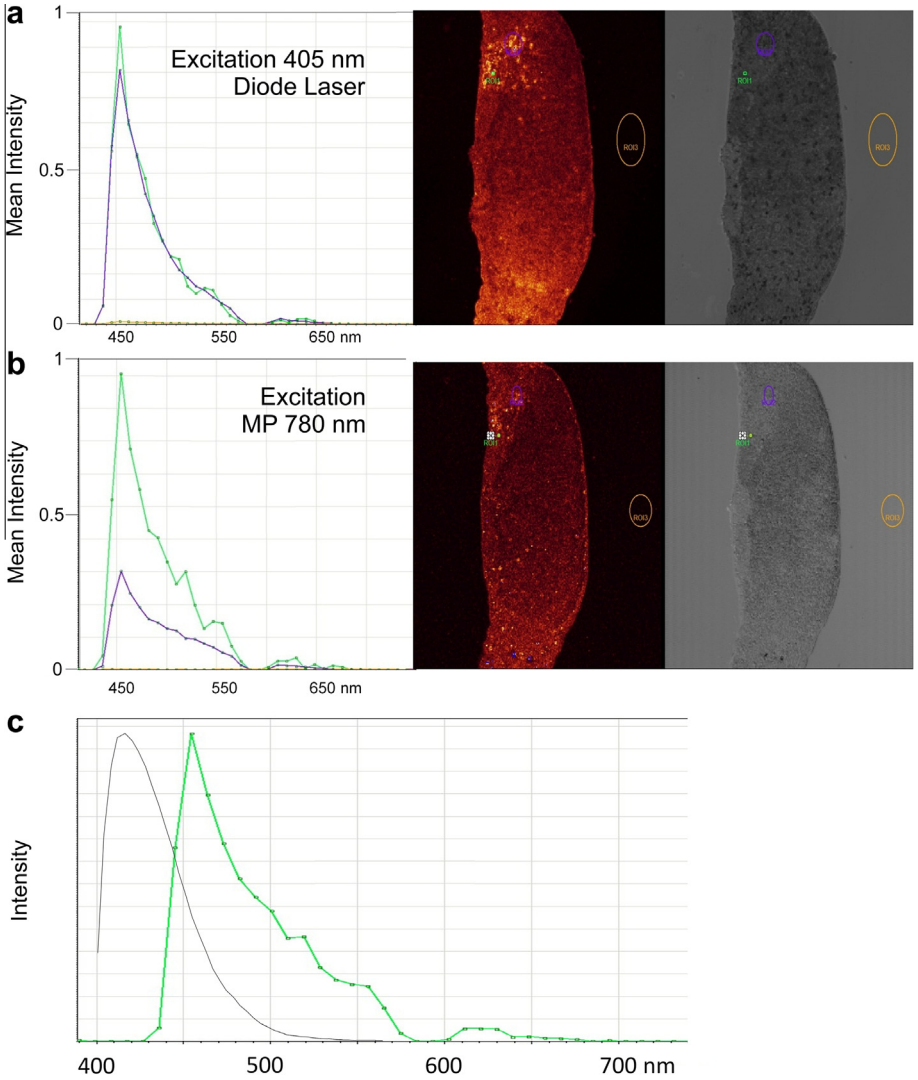
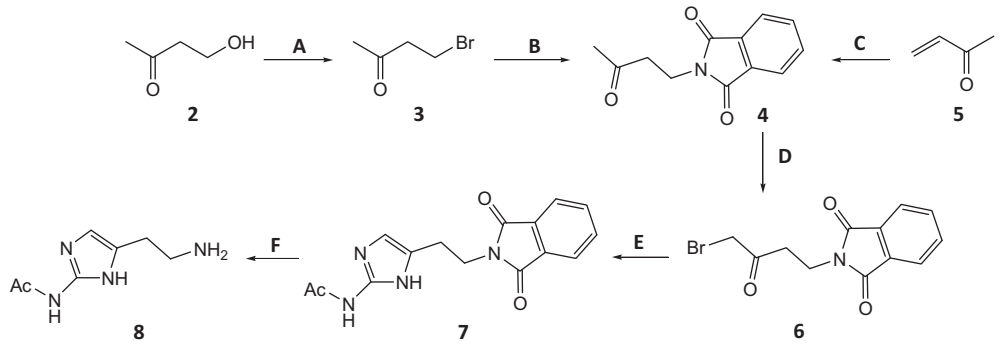
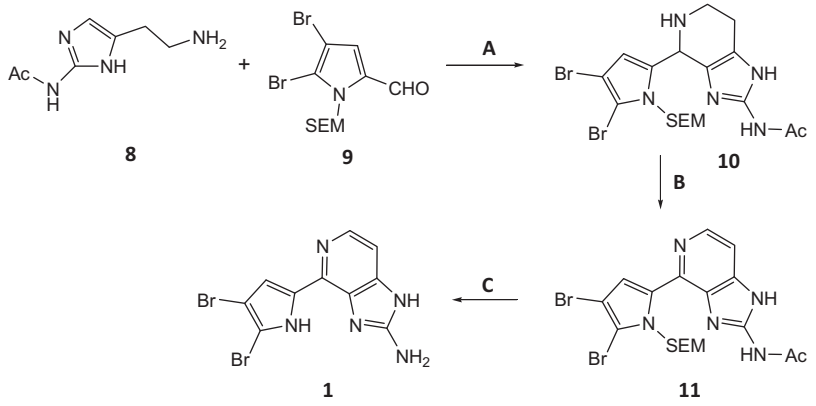


Figure 2. Emission spectra of three regions of interest during excitation with a 405 nm diode laser (a) and during excitation with a multiphoton laser at 780 nm (b). One region is outside of the animal (*Macrostomum lignano*) the others are inside of the animal. Emission spectra of published data of ageladine A in water[3] (**c**, black line), compared to the emission spectrum revealed inside a living flatworm (green line). There is a shift of the maximum amplitude of around 30 nm and the spectrum is broader, especially at longer wavelength.



Scheme 1. Synthesis of 2-amino-histamine: (A) PBr_3, pyridine, $CHCl_3$, 5 h, rt, 89%; (B) phthalimide potassium salt, DMF, 100 h, rt, 87%; (C) phthalimide, sodium ethanolate, ethanol, ethyl acetate, 2 h, rt then 3 h, 70 °C, 89%; (D) bromine, methanol, 30 min rt then 1.5 h 60 °C, 99%; (E) acetyl-guanidine, DMF, 100 h, rt, 70%; (F) (1) $NaBH_4$, 2-propanol/water, 24 h, rt (2) acetic acid, 2 h, 80 °C, 62% (both steps).



Scheme 2. Synthesis of ageladine A: (A) TEA, ethanol, 6 h, rt, 44%; (B) MnO_2, pyridine, acetone, 18 h, 70 °C, 56%; (C) ethanolic hydrochloric acid, 5 h, 80 °C, 71%.

Living tissue and especially membranes are made up of phospholipids. Many polar and unipolar molecules are present in living cells. As a result of this heterogeneous environment the emission profile may shift to a longer wavelength in the marine flatworm.

Figure 2a and b shows the increased Stokes shift of ageladine A in a living marine flatworm (*Macrostomum lignano*) compared to water (Fig. 2c). The emission maximum shifts 30 nm from about 420 nm to 450 nm and the emission profile is much broader, exceeding 550 nm. The comparison of a 405 nm diode laser to multiphoton excitation using 780 nm revealed no difference as could be expected as emission profiles do not change with excitation wavelength,[29] but with the environment. The altered Stokes shift in living tissue matches the filter settings for the commercially applied dye DAPI.

Experimental section

Fluorescence measurements using *Macrostomum lignano*

The culture of *Macrostomum lignano*[30] was originally received from Dita Vizoso and Lucas Schärer (Basel) and was raised and maintained in 16/8 LD cycle in Petri dishes together with the diatom *Nitzschia* sp. at 20 ± 2 °C in our lab since 2011. Worms were incubated in F/2 medium for 1 h with ageladine A, washed with medium, and anesthetized with 7.18% $MgCl_2$.

Fluorescence was monitored with a confocal laser scanning microscope TCS SP5 (Leica, Wetzlar, Germany) equipped with a multiphoton laser and other standard lasers. Wavelength scans were performed from 390 nm to 750 nm with a slit width of 10 nm (MP-excitation) or 420 nm to 750 nm for 405 nm diode laser excitation.

Synthesis

We characterized all synthesized compounds by NMR and/or MS. NMR spectra were recorded at 300 K and standard parameters. A Bruker Avance 400 MHz spectrometer at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) was used. High resolution mass spectra were recorded by a direct injection ESI-TOF mass spectrometer (Bruker micrOTOF, Bremen, Germany). All of the synthetic work was carried out using standard laboratory equipment. Chemicals were used without prior purification, except methanol and ethanol which were dried with sodium and distilled off.

Conclusions

The presented synthetic path offers the opportunity to produce ageladine A in gram-scale batches with standard laboratory equipment and making use of non-specialized chemicals. Using column-chromatography when needed, the only limitation is the volume of solvent that has to be used in some of the reaction steps (both brominations). The overall yield (starting with methyl vinyl ketone) is 6.6% over 7 steps (Schemes 1 and 2). The overall yield for the end-sequence (Scheme 2) is 18.0% (3 steps).

A comparison of our yields with the related and previously published yields of synthetic strategies by Ando and Terashima (11.7%, 9 steps)[14,31,32] Ma et al. (10.0%, last 3 steps)[15] and Karuso and Shengule (28.6%, last 3 steps)[16] reveal an average result.[33]

Due to our observations of yields and the purities of products no reaction steps were combined, while the work-up process is not necessary after every reaction. Chances to shorten this synthesis are given by combining steps A and B or B and C (both Scheme 2).[2]

The altered Stokes shift in living marine flatworms offers the opportunity to use common filter settings as, for example, DAPI filter to take advantage of the broad and shifted emission spectrum. Ageladine A therefore is useful in staining whole animals and dissected tissues.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.05.081. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; Yamashita, J.; van Soest, Rob W. M.; Fusetani, N. *J. Am. Chem. Soc.* **2003**, *125*, 15700–15701.
2. Shengule, S. R.; Loa-Kum-Cheung, W. L.; Parish, C. R.; Blairvacq, M.; Meijer, L.; Nakao, Y.; Karuso, P. *J. Med. Chem.* **2011**, *54*, 2492–2503.
3. Bickmeyer, U.; Grube, A.; Klings, K. W.; Köck, M. *Biochem. Biophys. Res. Commun.* **2008**, *3*, 419–422.
4. Hong-Hermesdorf, A.; Miethke, M.; Gallaher, S. D.; Kropat, J.; Dodani, S. C.; Chan, J.; Barupala, D.; Domaille, D. W.; Shirasaki, D. I.; Loo, J. A.; Weber, P. K.; Pett-Ridge, J.; Stemmler, T. L.; Chang, C. J.; Merchant, S. S. *Nat. Chem. Biol.* **2014**, *10*, 1034–1042.
5. Obermann, D.; Bickmeyer, U.; Wägele, H. *Toxicon* **2012**, *60*, 1108–1116.
6. Bickmeyer, U. *Mar. Drugs* **2012**, *10*, 223–233.
7. Bickmeyer, U.; Heine, M.; Podbielski, I.; Münd, D.; Köck, M.; Karuso, P. *Biochem. Biophys. Res. Commun.* **2010**, *402*, 489–494.
8. Bickmeyer, U.; Tietje, K.; Hofbauer, B.; Fink, C.; Roeder, T.; Schramm, G. *Procd. Göttingen Meeting German Neurosci. Soc.* **2013**. T27-6B.
9. Tietje, K.; Rivera-Ingraham, G.; Petters, C.; Abele, D.; Dringen, R.; Bickmeyer, U. *Mar. Drugs* **2013**, *11*, 3951–3969.
10. Meketa, M. L.; Weinreb, S. M. *Org. Lett.* **2007**, *9*, 853–855.
11. Meketa, M. L.; Weinreb, S. M. *Org. Lett.* **2006**, *8*, 1443–1446.
12. Mineno, T.; Kansui, H.; Yoshimitsu, H. *Tetrahedron Lett.* **2011**, *52*, 3131–3132.
13. Ando, N.; Terashima, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4495–4499.
14. Ando, N.; Terashima, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5461–5463.
15. Ma, Y.; Nam, S.; Jove, R.; Yakushijin, K.; Horne, D. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 83–86.
16. Shengule, S. R.; Karuso, P. *Org. Lett.* **2006**, *8*, 4083–4084.
17. Jones, R. C.; Kornpel, E. C.; Laughlin, K. C. *J. Am. Chem. Soc.* **1950**, 4526–4529.
18. Roquette, P. Ph.D. Dissertation, University of Heidelberg 2010, p 180f.
19. Ha, H.-J.; Lee, S.-K.; Ha, Y.-J.; Park, J.-W. *Synth. Commun.* **1994**, *24*, 2557–2562.
20. Beliaev, A.; Wahnon, J.; Russo, D. *Org. Process Res. Dev.* **2012**, *16*, 704–709.
21. Li, N.; Chu, X.; Liu, X.; Li, D. *Bioorg. Chem* **2009**, *37*, 33–40.
22. Dubief, R.; Robbe, Y.; Fernendez, J.-P.; Subra, G.; Terol, A.; Chapat, J.-P.; Sentenac-Roumanou, H.; Fatome, M. Eur. J. Med. Chem. - Chim. Ther. 1986, 6, 461–466.
23. Little, T. L.; Webber, S. E. *J. Org. Chem.* **1994**, *59*, 7299–7305.
24. Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093–2096.
25. Ilovich; O.; Deutsch, J. J. Heterocycl. Chem. 2005, 42, 1409–1411.
26. Anderson, H. J.; Lee, S.-F. *Can. J. Chem.* **1965**, *43*, 409–414.
27. Mordhorst, T.; Awal, S.; Jordan, S.; Petters, C.; Sartoris, L.; Dringen, R.; Bickmeyer, U. *Mar. Drugs* **2015**, *13*, 920–935.
28. Shengule, S. R.; Karuso, P. W. O. *Patent* **2009**, *2009152584*, A1.
29. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer Science + Business Media, LLC.: Berlin, 2006.
30. Ladurner, P.; Schärer, L.; Salvenmoser, W.; Rieger, R. M. *J. Zool. Syst. Evol. Res.* **2005**, *43*, 114–126.
31. Ando, N.; Terashima, S. *Synlett* **2006**, *17*, 2836–2840.
32. Ando, N.; Terashima, S. *Tetrahedron* **2010**, *66*, 6224–6237.
33. Because of the non-related synthetic strategy, all total-syntheses of ageladine A without a Pictet–Spengler reaction remain out of consideration.