RESEARCH ARTICLE doi.org/10.1002/adsc.202300560 Advanced Synthesis & Catalysis

From Pyridine to (−)-Agelastatin A

João R. Vale,[+a, b] Milene A. G. Fortunato,[+a] Késsia H. S. Andrade,[a] Ângelo M. R. Rocha,[a] Carlos A. M. Afonso,[a,*] and Filipa Siopa[a,*]

[a] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal
E-mail: carlosafonso@ff.ulisboa.pt; filipasiopa@ff.ulisboa.pt
[b] Faculty of Engineering and Natural Sciences, Tampere University, Korkeakoulunkatu 8, 33101 Tampere, Finland

[+] Both authors contributed equally to this manuscript.

Manuscript received: May 28, 2023; Version of record online: July 4, 2023

Supporting information for this article is available on the WWW under https://doi.org/10.1002/adsc.202300560

Abstract: (−)-Agelastatin A was synthetized employing a flow photorearrangement of a pyridinium salt, constructing in one step the cyclopentene core possessing the desired functionalities and relative configurations. A flow enzymatic kinetic resolution of the resulting bicyclic vinyl aziridine delivered the enantiopure precursor to the natural product. This total synthesis required the use of a single protective group. Two novel agelastatin *N3*-derivatives were synthesized and their cytotoxicity evaluated against a series of cancer cell lines, which corroborated the importance of unsubstituted *N3* in the biological activity of (−)-agelastatin A.

Keywords: total synthesis; natural product; photochemistry; flow chemistry; enzymatic resolution

Introduction

Agelastatin alkaloids (Figure 1), from the pyrrole-2-aminoimidazole alkaloid family, have attracted scientific interest since the isolation of (−)-agelastatin A (AglA) and B from the sponge *Agelas dendromorpha* by Pietra *et al.* in 1993.[1,2] Agelastatins C and D were later isolated and identified from *Cymbastela sp.* by Molinski *et al.*,[3] followed by agelastatins E and F, two minor metabolites isolated from *A. dendromorpha* by Al-Mourabit *et al.*[4]

AglA showed remarkable cytotoxicity against tumour cells, which prompted its intensive research.[5–7] In addition, AglA strongly inhibits osteopontin-mediated neoplastic transformation and metastasis,[8] heavily implicated in cancer progression, and displays high brine shrimp toxicity and insecticidal properties.[3] The structure-activity relationship (SAR) of (−)-AglA has been extensively studied and most modifications result in abrupt loss of anticancer activity (Figure 1). Only substitution on the pyrrole ring is tolerable, which has

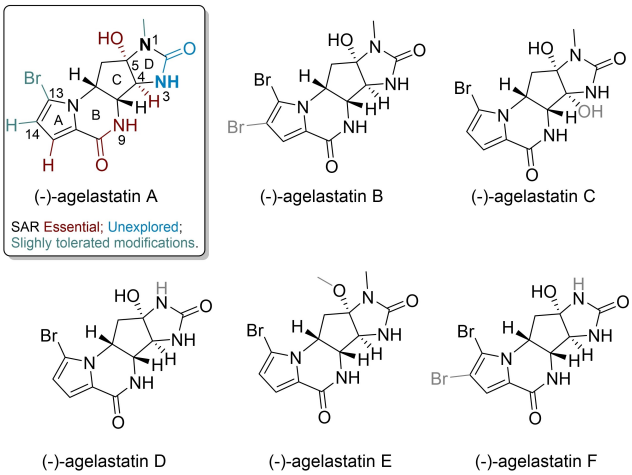


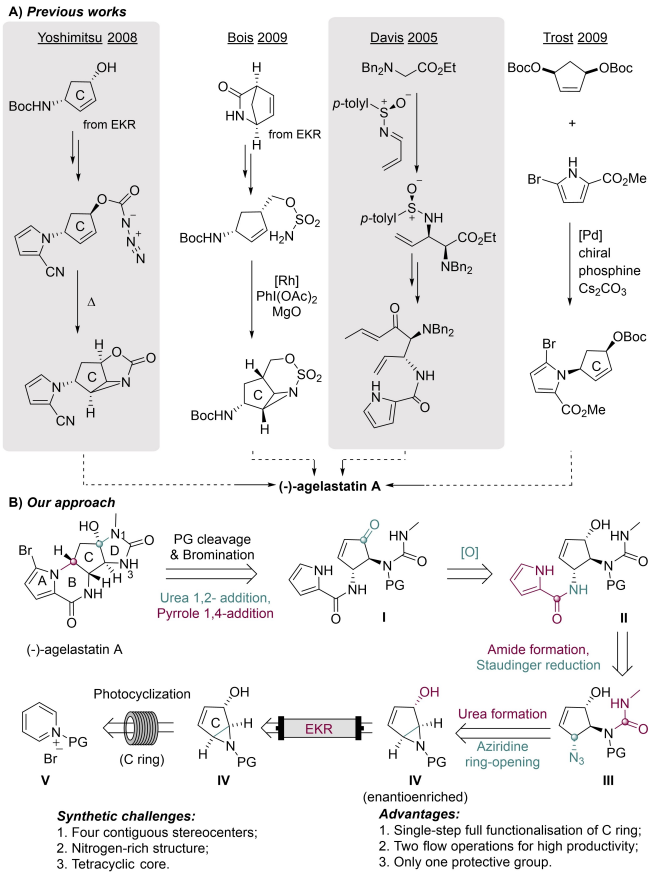
Figure 1. Natural agelastatin alkaloids. Known SAR of (−)-AglA is represented. (−)-agelastatins structural differences are highlighted in grey.

led to the discovery of two derivatives with better activity than (−)-AglA in some cancer cell lines: 13-chloro-agelastatin A,[9] and 13-trifluoromethyl-agelastatin A.[10]

The medicinal potential of AglA and the synthetic challenge of its nitrogen-rich tetracyclic core have prompted several total synthesis.[11] Concerning to racemic synthesis,[12–16] Batey and collaborators achieved in 2013 the shortest total synthesis of (±)-AglA in only six manipulations.[15] Starting with furfural, the key cyclopentenone intermediate comprising the C ring was synthesized *via* a thermal conrotatory π4a electrocyclization of a Stenhouse salt. The main challenge consisted in differentiating two identical primary amines after allylic deprotection. Extensive investigation on the most suitable amide coupling reagent revealed that TPTU allows regioselective transformation of the desired amine.

Asymmetric total synthesis of AglA is challenging and requires laborious steps to construct the four contiguous stereocenters in the cyclopentane C-ring. Since only the (−)-AglA enantiomer exhibits biological activity,[10] most efforts have been dedicated to its synthesis. Asymmetry is often achieved via enzymatic kinetic resolution (EKR) in early stages of the synthesis[17–20] or starting with biomass derived chiral precursors.[21–24] Selected examples of the success of EKR include the work of Yoshimitsu's group in 2008 (Scheme 1A), which employed it to obtain an amino cyclopentenol in excellent enantiomeric excess (*ee*), providing the initial core of the natural product.[18] Further functionalization was achieved via an intramolecular acyl nitrene aziridination and sequential ring-opening with azide. Similarly, Bois and collaborators in 2009 relied on a bioresolution of a γ-lactam with lactamase and functionalized the C-ring via rhodium catalysed oxidative aziridination and subsequent regioselective ring-opening with azide (Scheme 1A).[19] Alternative approaches to induce chirality include the work of Davis and co-workers, that in 2005 employed a diastereoselective enolate addition to a chiral sulfonimine (Scheme 1A);[25] and the work of Trost's group, that in 2009 took advantage of their asymmetric allylic substitution methodology in the desymmetrization of a *meso* allylic dicarbonate via addition with a pyrrole nucleophile (Scheme 1A).[26]

Our synthetic strategy was inspired in the highly functionalised cyclopentene ring **IV** (Scheme 1B). We envisioned that ring-opening of its aziridine with azide would provide the *trans*1,2-diamine motif with chemically differentiated nitrogens. In addition, the presence of a secondary alcohol in **IV** could allow novel EKR to introduce chirality in three contiguous centres at an early stage. Enantioenriched **IV** presents the cyclopentane C-ring containing the desired functionalities and absolute configuration of vital chiral centres to allow facile access to (−)-AglA. Taking this into account, retrosynthetic analysis of (−)-AglA was designed (Scheme 1B). The tetracyclic core was deconstructed via sequential 1,4-addition of the pyrrole nitrogen of **I** onto the enone, forming B ring, and subsequent hemiaminal formation via 1,2-addition of the urea to the resulting ketone, forming D ring. Intermediate **I** would result from oxidation of allylic alcohol **II**. The amide bond containing the pyrrole moiety in **II** would be accessible from Staudinger reduction of allylic azide **III** and sequential acylation. We planned to construct **III** by urea formation on the secondary amine, which traces back to the bicyclic vinyl aziridine **IV** after ring-opening with azide. Aziridines **IV** are obtained via photoirradiation of pyridinium salts **V**.[27,28] Our experience with the synthesis of **IV** via photoirradiation of pyridinium salts **V** alerts at the importance of careful protecting group selection.[29,30] The photo transformation of pyridinium salts is not compatible with common amine protecting electron withdrawing groups and benzylic protection is low yielding due to competitive absorption of UV light. Ultimately, we decided to use allyl group given the abundance of methods for its deprotection[31] and its compatibility with the photochemical reaction.[32]

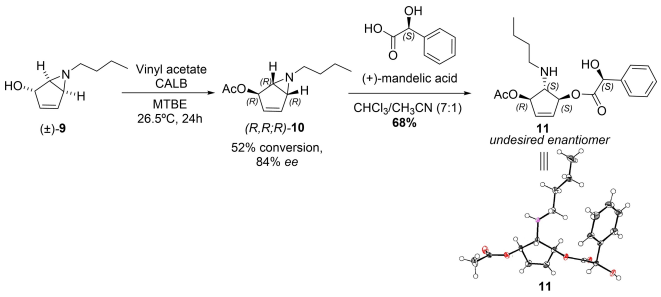


Scheme 1. (A) Selected examples of total synthesis of (−)-agelastatin A. (B) Our retrosynthetic approach for the synthesis of (−)-agelastatin A.

Results and Discussion

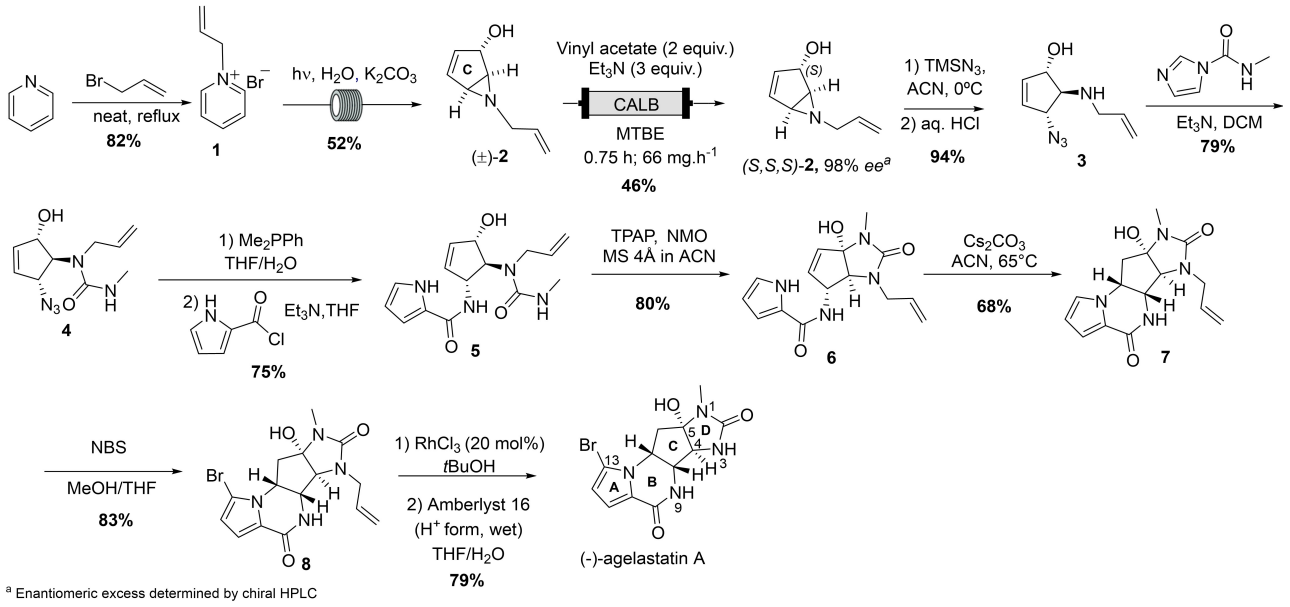
Our synthetic studies were first focused on the photochemical synthesis in flow of (±)-**2** (Scheme 2). Bicyclic aziridine (±)-**2** was obtained following our reported procedure with a productivity of 129 mg.h^{-1}.[33] We then explored the EKR of (±)-**2** using CALB (Novozym 435), an immobilized lipase, efficient for enantioselective alcohol acetylations.[34–36] Preliminary investigations on the enzymatic acetylation used as a model substrate butyl aziridine (±)-**9** (Scheme 3). Following EKR, suitable crystals for X-ray analysis (see the Supporting information for details) could be obtained by ring opening reaction of enantioenriched acetyl butyl bicyclic aziridine **10** with (+)-mandelic acid, which revealed that CALB preferentially acetylates the undesired enantiomer for the synthesis of the target natural product (Scheme 3).

The resolution of (±)-**2**, was performed under flow EKR to obtain the desired enantioenriched (*S,S,S*)-**2** (from now referred to as (*S*)-**2**) in suitable scale for the total synthesis of (−)-AglA (Scheme 2). During this process, partial hydrolysis of acylating agent resulted in the undesired nucleophilic ring-opening of (±)-**2** with acetic acid. After optimization, we observed that the side reaction could be prevented by adding Et_3N to the reaction mixture (see the Supporting information for details). Changing the residence time from 45 min to 10 min, it was possible to obtain either highly enantioenriched (*S*)-**2** or the acetylated *R* product, respectively. The resolution of (±)-**2** was intensified via a longer enzymatic reactor (see the Supporting information for details), which allowed to resolve 66 mg.h^{-1} of (±)-**2**, resulting in isolation of (*S*)-**2** in 98% *ee* and 46% yield (Scheme 2). Flow EKR proved also suitable for the resolution of (±)-**9**, allowing to obtain the (*R,R,R*)-**10** with 95% *ee* and conversions of 46% (see the Supporting information for details).



Scheme 3. Identification of the absolute stereochemistry of enriched acetylated butyl bicyclic aziridine (*R*)-**10**.

The enantioenriched aziridine (*S*)-**2** was subjected to ring opening with $TMSN_3$ to give **3** (Scheme 2) in 94% yield. As observed for other nucleophiles, aziridines such as (*S*)-**2** undergo S_N2 ring-opening selectively on the allylic carbon of the aziridine.[29] Then, urea formation was accomplished in 79% yield (**4**), following Batey procedure using *N*-methyl carbamoylimidazole,[37] without competitive attack of the alcohol. Attempts to reduce the azide **4** with PPh_3 consistently gave a major unidentified side product, possibly a stable iminophosphorane unreactive towards acyl chlorides. Staudinger azide reduction with Me_2PhP or nBu_3P, cleanly provided primary amine intermediate which was acylated without isolation with freshly prepared pyrrole-2-carbonyl chloride, providing intermediate **5** in good 75% yield (Scheme 2).



Scheme 2. Total synthesis of (−)-agelastatin A.

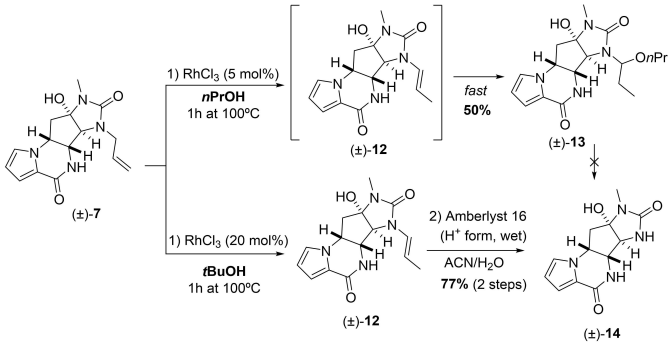
We then explored conditions for a tandem alcohol oxidation followed by urea 1,2-addition. Swern conditions, IBX, PCC or MnO_2 were investigated. All methods invariably led to incomplete conversions, even with the use of large excess of oxidising agents. Satisfyingly, Ley-Griffith oxidation led to nearly full conversion in short reaction time, allowing isolation of cyclic hemiaminal **6** in good yield (80%) (Scheme 2).

We considered to obtain the final nitrogen-carbon bound of tetracyclic AglA core through formation of a transient enone trapped in situ by the pyrrole. Heating **6** in the presence of Cs_2CO_3 in acetonitrile delivered the desired tetracyclic **7** in 68% yield. Presumably, the carbonate base deprotonates the hemiaminal, shifting the equilibrium to the ketone form while cesium acts as Lewis acid activating the enone. From there, pyrrole bromination with NBS yielded the desired product **8** and as minor side product its *C*14-bromo regioisomer, inseparable from **8**. Late-stage bromination of the agelastatin scaffold is known to give as minor impurity the *C*14-bromo regioisomer which needs to be removed via semi-preparative or preparative HPLC methods.[10] In our case, the use of excess NBS was successful in completely dibrominating the minor undesired *C*14 regioisomer. The dibromo derivative was then separable from **8** by standard preparative silica chromatography, allowing isolation of **8** in 83% yield.

Cleavage of the allyl moiety is the final transformation required to obtain (−)-AglA. Racemic (±)-**7** was used as model compound to study the deallylation reaction. Although allylic deprotection through palladium catalysis is a straightforward approach for allylamines,[38] deprotection of allylic amides and ureas is more challenging. In fact, palladium catalysed deallylation, using 1,3-dimethylbarbituric acid[38] or formic acid/triethylamine[39] as scavengers, lead to recovery of starting material (±)-**7**. DDQ oxidative cleavage of the allyl group[40] also failed, leading to a complex mixture of products after prolonged reaction times at high temperatures. Formation of a transient enamine has been used with limited success by Bennet's group[32] to deprotect an allylic amide from a similar precursor. We screened conditions that would promote isomerisation of the allylic urea (±)-**7**. Transition metal catalysis with Ru(II)[41] and Fe_2CO_9[42] were tested but unsuccessful. For allylic amides specifically, $RhCl_3$ catalysis in alcoholic solvents such as *n*-propanol has been used in the literature as an efficient deprotection method.[43] Presumably, a Rh(I) hydride specie is formed through solvent oxidation, and acts as catalyst in the isomerization of the allyl urea to a more thermodynamically stable vinyl urea. The catalytic HCl, that is formed after solvolysis of $RhCl_3$, then promotes the transformation to the hemiaminal ether which is later cleaved to deliver the allyl-deprotected product. In our hands, refluxing (±)-**7** in *n*-propanol in the presence of 5 mol% of $RhCl_3$ resulted in complete consumption of the starting material after one hour. However, the reaction halted at the hemiaminal ether stage ((±)-**13**), which could be isolated in 50% yield (Scheme 4). Although a possible intermediate in the deallylation sequence, this product failed to give the desired deprotected compound (±)-**14** through longer reaction times or under acidic aqueous conditions. Addition of water to the rhodium catalysed reaction envisioning the formation of a free hemiaminal lead to no conversion.

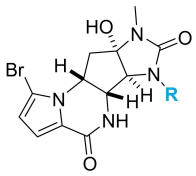
Finally, replacing *n*-propanol solvent with *t*-butanol as non-nucleophilic solvent completely prevented hemiaminal ether formation (±)-**13** (Scheme 4), yielding the vinyl urea (±)-**12**. $RhCl_3$ catalyst loading of 20 mol% was required to assure complete conversion of the starting material. Compound (±)-**12** was prone to hydrolysis under acidic aqueous conditions such as gently warming in presence of acidic Amberlyst, efficiently converting it to (±)-**14**. Application of this modified procedure to compound **8** (Scheme 2) successfully delivered (−)-AglA in 79% yield.

After developing the total synthesis of (−)-AglA we focused our attention on the synthesis of novel *N3*-substituted AglA derivatives as the effect of *N*3-substituition on the biological activity of (−)-AglA is not completely clear (Figure 1). Literature analysis revealed that the only two reported *N*3-alkylated (methylated) AglA derivatives evaluated were inactive but also presented methylation in *N*9 or *O*5, modifications known to drastically reduce agelastatin activity.[5,44] According to the crystal X-ray structure of AglA bound to the active site, *N3* does not seem to be interacting with the ribosome,[45] which suggests that substitution at this position could provide new agelastatin derivatives without compromising their biological activity. Our novel synthetic pathway allows preparation of *N3*-substituted AglA by changing the group installed on the pyridinium ion, creating the opportunity to study the effect of *N3*-substituition on the biological activity of AglA derivatives. Starting with butyl pyridinium salt, we prepared *N3*-butyl AglA derivative (±)-**15**, following the implemented synthetic pathway towards (−)-



Scheme 4. Allyl deprotection with catalytic RhCl3 during (±)-AglA total synthesis.

Table 1. Cytotoxicity of AglA Derivatives against MDA, HCI–H460 and HCT 116 cancer cell lines.

Compound	IC_{50} (μM)		
	MDA	NCI–H460	HCT 116
(±)-**8**, R=Allyl	>50	>50	>50
(±)-**15**, R = *n*-Butyl	>50	23.5±4.1	>50
(±)-AglA, R=H	5.6±2.1	2.6±0.4	4.0±0.4

AglA (see the Supporting information for details). The cytotoxicity of racemic *N3*-allyl AglA (±)-**8**, *N3*-butyl AglA (±)-**15** and (±)-AglA was evaluated against human breast cancer cells line (MDA-MB-231), human colorectal cancer cells line (HCT-116) and human lung cancer cells line (NCI-H460) by a Neutral red cell cytotoxicity assay (Table 1). (±)-AglA presented cytotoxic activity in the micromolar range for the three cell lines tested.[9,46] However, for the two *N3*-alkylated derivatives (±)-**8** and (±)-**15**, the activity was completely lost, showing the importance of unsubstituted *N3* in the bioactivity of the natural product.

Conclusion

We have developed a novel asymmetric synthesis of the natural alkaloid (−)-AglA. This synthesis starts from cheap and easily accessible starting materials and the challenging C ring is constructed at early-stage, with the required relative stereochemistry and functional groups already installed. The aziridine photochemical production was achieved in multi-gram scale under continuous flow. The asymmetric version was attained by utilizing the cheap and practical CALB lipase (Novozym® 435) via a second flow approach. The highly active natural product was obtained in 12 steps (10 operations) and 4% overall yield. Notably, the sequence required the use of a single protecting group, a requirement of the initial photochemical transformation of pyridinium salt. Our new synthetic methodology proved to be robust by enabling the preparation of *N3*-substituted AglA derivatives. Specifically, (±)-*N3*-butylagelastatin ((±)-**15**) was obtained in 23% overall yield starting with butylation of pyridine. The direct effect of *N3*-substitution on the biological activity of AglA derivatives was evaluated for the first time. We were able to confirm that *N3*-alkylation of AglA hinders its cytotoxicity against cancer cell lines.

Experimental Section

General Procedure for Synthesis of Pyridinium Salts

To an Aldrich ACE pressure tube (Z181064) at room temperature were added pyridine (5.1 mL, 0.06 mol) and the respective brominated derivative (0.06 mol). The solution was heated at 60 °C overnight, unless otherwise mention. After cooling, the salt was dissolved in MeOH, and the solution was transferred to a flask and evaporated under vacuum to obtain the pure pyridinium salt.

1-Allylpyridinium Bromide (1) was obtained in 82% yield (9.8 g, 0.05 mol) as a brown solid. **^{1}H NMR** (300 MHz, D_2O) δ 9.00 (d, J=5.5 Hz, 2H), 8.72–8.67 (m, 1H), 8.22 (t, J=7.0 Hz, 2H), 6.34–6.21 (m, 1H), 5.67–5.59 (m, 2H), 5.37 (d, J=6.0 Hz, 2H). The spectral data are in accordance with the literature.[47]

1-n-Butylpyridinium Bromide (18) the solution was heated at 100 °C for 40 h, and **18** was obtained in quantitative yield solid (13.0 g, 0.06 mol) as a colourless solid. **^{1}H NMR** (300 MHz, D_2O) δ 8.93 (d, J=6.1 Hz, 2H), 8.65–8.59 (m, 1H), 8.14 (t, J=7.1 Hz, 2H), 4.69 (t, J=7.4 Hz, 2H), 2.12–2.02 (m, 2H), 1.47–1.37 (m, 2H), 1.03–0.98 (m, 3H). The spectral data are in accordance with the literature.[47]

General Procedure for Synthesis of Bicyclic Aziridines

Prepared according to the procedure reported by us[33] a parallel quartz tube (PQT6) reactor consisting of 12 QT6 tubes (length: ±100 cm; 95 cm under irradiation; internal diameter: 0.6 cm) was placed inside the home-made UV reactor. To fill the PQT6, a 350 mL of an aqueous solution of the corresponding pyridinium salt (20 mM) and potassium carbonate (1.2 molar equiv.) was pumped using the multichannel cassette pumps Watson Marlow 205S (12 silicon tubes 1×3 mm). Then, the pump was set to 3 rpm (0.12 mL/min flow rate) and the irradiation was turned on. An aqueous solution of pyridinium salt (20 mM) and potassium carbonate (1.2 molar equiv.) was pumped (0.12 mL/min flow rate). To isolate the bicyclic aziridine the water corresponding to a certain period of irradiation was evaporated under vacuum and the obtained solid was dissolved in dichloromethane (3× 250 mL), stirred for 15 min, and filtered. The solvent was evaporated to give the pure bicyclic aziridine.

6-Allyl-6-azabicyclo[3.1.0]hex–3-en-2-ol (2), 1360 mL of collected water corresponding to the period of 15 h of irradiation, obtaining 1.9 g (52%) of **2** as a brown oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.32–6.29 (m, 1H), 5.95–5.86 (m, 2H), 5.23–5.19 (m, 2H), 4.52 (s, 1H), 3.02–3.27 (m, 2H), 2.56–2.54 (m, 1H), 2.51 (dd, J=4.3, 1.9 Hz, 1H). The spectral data are in accordance with the literature.[47]

6-n-Butyl-6-azabicyclo[3.1.0]hex–3-en-2-ol (9), 6515 mL of collected water corresponding to the period of 42.7 h of irradiation, obtaining 17.0 g (85%) of **9** as a brown oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.29–6.27 (m, 1H), 5.88–5.86 (m, 1H), 4.48 (d, J=1.2 Hz, 1H), 2.48–2.47 (m, 1H), 2.44–2.43 (m, 1H), 2.37–2.19 (m, 2H), 1.60–1.50 (m, 2H), 1.41–1.31 (m, 2H), 0.89 (t, J=7.3 Hz, 3H). The spectral data are in accordance with the literature.[47]

EKR in Flow of (±)-2

A packed bed reactor of Teflon (internal volume of 3 mL, length 460 mm, ø = 4 mm) filled with a mixture of CALB immobilized (Novozym® 435 beads, 1.45 g) and sand (neutralized sand in a solution of 5% Et_3N, 3.6 g), was placed in an incubator at 26.5 °C, and MTBE (3–4 volumes of the reactor) was pumped, in upward direction, to wet the beads. The collecting vessel is on a bath bellow 0 °C. Aziridine (±)-**2** (204 mg, 1.49 mmol, ratio 0.034 mg $_{aziridine}$/mg $_{enzyme}$) was dissolved in a solution of 12 mL of MTBE with naphthalene as internal standard ([naphthalene] = 0.3 mg/mL). Triethylamine (3 equiv., 4.47 mmol) and vinyl acetate (2 equiv., 2.98 mmol) were added to the solution, and it was injected in the continuous flow reactor with a residence time of 45 minutes. Samples were analysed by GC and/or HPLC. Crude reactions (560.5 mg of starting material) were set together and purified by silica column chromatography (eluent: ethyl acetate) to give 260 mg of (*S,S,S*)-**2** (*ee* 98%, isolated yield 46%).

Synthesis of (−)-Agelastatin A from Enantioenriched 2

(3): Enantioenriched aziridine (*S,S,S*)-**2** (182 mg, 1.33 mmol) was dissolved in 4 mL of ACN in a round-bottom flask. Solution was cooled to 0 °C and $TMSN_3$ (6.6 mL, 4 mmol, 3 equiv.) was added dropwise and the solution was stirred during 4 hours at room temperature. The solvent was evaporated, and 5 mL of aqueous HCl (1 M) was added and the solution was left stirring for 5 minutes. Then, the mixture was basified with aqueous saturated Na_2CO_3. 5 mL of EtOAc was added, the mixture was transferred to an extraction funnel and the layers were separated. The organic solvent was collected. The aqueous phase was extracted with EtOAc (3 × 5 mL). The organic phases were combined and dried over $MgSO_4$, filtered and evaporated to give pure azide **3** as a brown oil in 94% yield (226 mg, 1.25 mmol). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 5.99–5.81 (m, 3H), 5.28–5.13 (m, 2H), 4.49–4.45 (m, 1H), 3.96–3.94 (m, 1H), 3.50–3.34 (m, 2H), 3.13 (t, *J* = 5.1 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 136.8, 136.1, 130.1, 116.9, 80.7, 73.9, 70.3, 50.9. **HRMS *m/z***: $[M+H]^+$ calculated for $C_8H_{13}N_4O_2^+$197.1033, found 197.1031 (oxidised amine). **Note 1**: The HCl treatment is required to hydrolyse the silyl ether formed during the reaction and release the free alcohol. **Note 2**: Aziridine **2** is slightly unstable even with low temperature storage. If the sample of **2** presents degradation, the ring-opening reaction with azide is not clean. We suggest taking the crude of **3** and proceed to the next step to form **4**, and then purify **4** via silica column chromatography.

(4): Amine **3** (224 mg, 1.24 mmol, 1 equiv.) was dissolved in 4 mL of dry DCM in an argon purged round-bottom flask. Dry triethylamine (347 µL, 2.48 mmol, 2 equiv.) was added, followed by the imidazole derivative (310 mg, 2.48 mmol, 2 equiv.). The solution was stirred for 24 hours at room temperature. Reaction was quenched with 5 mL of saturated aqueous $NaHCO_3$. The mixture was transferred to an extraction funnel and the layers were separated. The organic solvent was collected. The aqueous phase was extracted with DCM (2 × 5 mL). The organic phases were combined and dried over $MgSO_4$, filtered and evaporated. The resulting crude oil was purified by silica column chromatography, eluent EtOAc (100%), to give urea **4** in 79% yield (233 mg, 0.98 mmol) as an oil. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.02–5.81 (m, 3H), 5.41–5.28 (m, 2H), 4.86–4.84 (m, 1H), 4.68–4.66 (m, 1H) 4.50 (dq, *J* = 6.6, 1.5 Hz, 1H), 3.97–3.76 (m, 3H), 2.78 (d, *J* = 4.7 Hz, 3H). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 160.0, 136.8, 134.1, 129.9, 117.3, 77.7, 74.5, 66.5, 50.4, 27.6. **HRMS *m/z***: $[M+H]^+$ calculated for $C_{10}H_{16}N_5O_2^+$238.12985, found 238.12960.

(5): Azide **4** (200 mg, 0.84 mmol) was dissolved it in 10 mL of THF/H_2O (9:1) mixture. Dimethylphenylphosphine (147 µL, 1.01 mmol, 1.2 equiv.) was added dropwise under a nitrogen atmosphere. After one hour, the solvents were evaporated under reduced pressure. The resulting oil was dissolved in 20 mL of DCM and the solution dried over $MgSO_4$, filtered, and evaporated. The residue was further dried in high vacuum for one hour. The crude was dissolved in 25 mL of dry THF, under an argon atmosphere. Dry triethylamine (236 µL, 1.67 mmol, 2 equiv.) was added and the solution cooled to 0 °C. Pyrrole-2-carbonyl chloride (109 mg, 0.86 mmol, 1.0 equiv.) was added in one portion at this temperature. The solution was stirred at 0 °C for 5 minutes and then left at room temperature for 24 h. The reaction was quenched with 30 mL of saturated aqueous $NaHCO_3$ and diluted with 10 mL of EtOAc. The mixture was transferred to an extraction funnel and the layers were separated. The organic solvent was collected. The aqueous phase was extracted with EtOAc (2 × 30 mL). The organic phases were combined and dried over $MgSO_4$, filtered and evaporated. The resulting crude was purified by silica column chromatography, eluent EtOAc (100%), to give amide **5** in 75% yield (193 mg, 0.63 mmol) as a white solid, MP 96–97 °C. **^{1}H NMR** (CD_3OD, 300 MHz) δ 6.91 (dd, *J* = 2.7, 1.4 Hz, 1H), 6.80 (dd, *J* = 3.8, 1.4 Hz, 1H), 6.16 (dd, *J* = 3.8, 2.5 Hz, 1H), 5.91–5.78 (m, 3H), 5.29–5.19 (m, 2H), 5.09 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.56 (s, 1H), 4.14 (t, *J* = 6.9 Hz, 1H), 4.04–3.81 (m, 2H), 2.70 (s, 3H). **^{13}C NMR** (CD_3OD, 75 MHz) δ 163.4, 161.2, 135.6, 135.5, 133.7, 126.7, 123.0, 116.8, 112.2, 110.2, 77.2, 74.8, 56.2, 49.9, 27.6. **HRMS *m/z***: $[M+H]^+$ calculated for $C_{15}H_{21}N_4O_3^+$305.1608, found 305.1606.

(6): Alcohol **5** (119 mg, 0.39 mol, 1 equiv.) was dissolved in 2.5 mL of dry acetonitrile, in an argon filled dry round-bottom flask. NMO (275 mg, 2.35 mmol, 6 equiv.) and 4 Å molecular sieves (195 mg, powdered) were added. TPAP (14 mg, 0.04 mmol, 10 mol%) was then added in one portion. After 20 min the solution was filtered through a small silica pad, which was washed with acetonitrile (2 × 5 mL). The crude was concentrated under reduced pressure. Purification through silica column chromatography, eluent EtOAc, gave hemiaminal **6** in 80% yield (95 mg, 0.31 mmol) as a white powder, MP 184–185 °C. **^{1}H NMR** (CD_3OD, 300 MHz) δ 6.92 (dd, *J* = 2.6, 1.4 Hz, 1H), 6.82 (dd, *J* = 3.8, 1.4 Hz, 1H), 6.22–6.15 (m, 2H), 5.95 (dd, *J* = 5.8, 2.4 Hz, 1H), 5.84–5.71 (m, 1H), 5.23–5.10 (m, 2H), 4.89–4.87 (m, 1H), 4.28–4.20 (m, 1H), 3.92 (dd, *J* = 15.5, 7.4 Hz, 1H), 3.68 (d, *J* = 2.0 Hz, 1H), 2.83 (s, 3H). **^{13}C NMR** (CD_3OD, 75 MHz) δ 162.8, 158.9, 134.9, 133.9, 133.5, 126.5, 123.2, 118.6, 112.3, 110.3, 98.1, 73.1, 60.6, 45.5, 25.6. **HRMS *m/z***: $[M+H]^+$ calculated for $C_{15}H_{19}N_4O_3^+$303.1452, found 303.1451.

(7): Allylic hemiaminal **6** (95 mg, 0.31 mmol, 1 equiv.) was dissolved in 9 mL of dry acetonitrile, in a argon filled dry round-bottom flask. Caesium carbonate (111 mg, 0.34 mmol, 1.1 equiv.) was added and the solution stirred at 65 °C for 4 hours. The

reaction was concentrated under reduced pressure and the product purified by silica column chromatography, eluent EtOAc:EtOH (94:6) to give **7** in 68% yield (64 mg, 0.21 mmol) as a white powder, MP 202–203 °C. **^{1}H NMR** (CD_3OD, 300 MHz) δ 7.01 (d, J = 1.8 Hz, 1H), 6.89 (dd, J = 3.9, 1.6 Hz, 1H), 6.27–6.19 (m, 1H), 5.89–5.76 (m, 1H), 5.32–5.20 (m, 2H), 4.65–4.55 (m, 1H), 4.14 (d, J = 5.4 Hz, 1H), 4.06 (dd, J = 15.3, 4.8 Hz, 1H), 3.84–3.79 (m, 1H), 3.76 (s, 1H), 2.83 (s, 3H), 2.63 (dd, J = 13.4, 6.4 Hz, 1H), 2.32 (dd, J = 13.3, 10.2 Hz, 1H). **^{13}C NMR** (CD_3OD, 75 MHz) δ 162.1, 159.7, 134.2, 125.6, 122.9, 118.7, 115.4, 111.1, 93.7, 71.7, 60.3, 55.7, 46.3, 41.6, 24.7. **HRMS *m/z***: $[M+H]^+$ calculated for $C_{15}H_{19}N_4O_3^+$303.1452, found 303.1451.

(8): Pyrrole **7** (8.3 mg, 27 µmol) was dissolved in 2 mL of MeOH/THF (1:2). The solution was cooled to 0 °C and freshly recrystallized NBS (6.4 mg, 0.036 µmol, 1.3 equiv.) in 0.5 mL of THF was added dropwise. After 10 minutes at 0 °C the solution was left warming to room temperature. The solvent was evaporated, and the crude purified via preparative TLC, eluent MeOH/EtOAC (0.4:99.6), 3 elutions, to give bromopyrrole **8** in 83% yield (8.5 mg, 22.2 µmol) as a white amorphous solid. **^{1}H NMR** (CD_3OD, 300 MHz) δ 6.91 (d, J = 4.1 Hz, 1H), 6.33 (d, J = 4.1 Hz, 1H), 5.91–5.78 (m, 1H), 5.33–5.21 (m, 2H), 4.59–4.51 (m, 1H), 4.24 (d, J = 5.5 Hz, 1H), 4.05 (dd, J = 15.8, 5.7 Hz, 1H), 3.83 (s, 1H), 3.83–3.76 (m, 1H), 2.84 (s, 3H), 2.67 (dd, J = 13.1, 6.5 Hz, 1H), 2.12 (t, J = 12.6 Hz, 1H). **^{13}C NMR** (CD_3OD, 75 MHz) δ 161.2, 159.9, 134.2, 124.2, 118.7, 116.0, 113.8, 107.2, 93.6, 71.2, 59.3, 54.4, 46.4, 40.0, 24.6. **HRMS *m/z***: $[M+H]^+$ calculated for $C_{15}H_{18}BrN_4O_3^+$381.0557, found 381.0560.

(−)-Agelastatin A: Allyl-agelastatin A **8** (8.5 mg, 22.2 µmol) was dissolved in 2.2 mL of dry *t*BuOH. Rhodium chloride was added (1.2 mg, 0.44 µmol, 0.2 equiv.) and the solution heated to 100 °C for 6 hours. The solvent was evaporated, and the crude redissolved in 2.2 mL of THF/H_2O (1:1). Then, 85 mg of Amberlyst® 16 wet (hydrogen form, strongly acidic) were added, and the solution was heated to 70 °C for 6 hours. The solution was filtered through celite, and the solvents evaporated. The crude was purified via silica column chromatography, eluent DCM/MeOH/NH_4OH (87:12:1) to give agelastatin A in 79% yield (5.9 mg, 17.3 µmol). **^{1}H NMR** (300 MHz, $(CD_3)_2SO$) δ 7.99 (s, 1H), 7.09 (d, J = 2.4 Hz, 1H), 6.73 (d, J = 4.0 Hz, 1H), 6.51 (s, 1H), 6.34 (d, J = 4.0 Hz, 1H), 4.40–4.32 (m, 1H), 3.96 (d, J = 5.4 Hz, 1H), 3.76 (d, J = 2.4 Hz, 1H), 2.64 (s, 3H), 2.46–2.42 (m, 1H), 1.92 (t, J = 12.5 Hz, 1H). **^{13}C NMR** (75 MHz, $(CD_3)_2SO$) δ 158.6, 157.7, 123.7, 113.3, 111.8, 104.5, 93.4, 65.1, 60.3, 52.5, 38.7, 23.5. Data in accordance with the literature.[1] **HRMS *m/z***: $[M+H]^+$ calculated for $C_{12}H_{14}BrN_4O_3^+$341.0244, found 341.0243.

EKR in Batch for (±)-9

Aziridine (±)-**9** (10 mg, 0.07 mmol) was dissolved in 1 mL of MTBE and 10 mg of CALB (Novozym® 435 beads) were added. Vinyl acetate (12.1 µL, 0.13 mmol, 2 equiv.) was then added to the solution, which was placed under mild stirring at 26.5 °C. After 24 hours the conversion of the reaction was 52%, (*S,S,S*)-**9** had 91.4% *ee* and (*R,R,R*)-**10** had 84.1% *ee*.

***6-butyl-6-azabicyclo[3.1.0]hex–3-en-2-ol (9)*: ^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.31–6.28 (m, 1H). 5.89–5.86 (m, 1H). 4.48 (d, J = 1.2 Hz,1H), 2.48–2.47 (m, 1H), 2.44–2.43 (m, 1H), 2.39–2.19 (m, 2H) 1.60–1.50 (m, 2H), 1.41–1.31 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H). The spectral data are in accordance with the literature.[30]

***6-butyl-6-azabicyclo[3.1.0]hex–3-en-2-yl acetate (10)*: ^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.38 (dt, J = 5.6, 1.4 Hz, 1H), 5.82–5.80 (m, 1H), 5.43 (t, J = 1.8 Hz, 1H), 2.53–2.46 (m, 2H), 2.39–2.21 (m, 2H), 2.07 (s, 3H), 1.57–1.52 (m, 2H), 1.38–1.32 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H). The spectral data are in accordance with the literature.[48]

Synthesis of (*2S*)-4-acetoxy-5-(butylamino)cyclopent-2-envyl-2-hydroxy-2-phenylacetate (11)

To a solution of acetylated aziridine (*R,R,R*)-**10** (32 mg, 0.16 mmol) in chloroform/acetonitrile (7:1) was added (*S*)-mandelic acid (0.029 g, 0.19 mmol). The suspension was stirred at room temperature for 48 hours. After concentration under reduced pressure, the mixture was purified by column chromatography (eluent: ethyl acetate/*n*-hexane/triethylamine (40:20:1)). The product was concentrated under reduced pressure and left to crystallize by slow evaporation of the solvent, to give the desired product **11** as white to pale yellow crystals (38 mg, 68%). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.40–7.35 (m, 2H), 7.29–7.17 (m, 3H), 5.85 (d, J = 6.0 Hz, 1H), 5.64 (d, J = 6.0 Hz, 1H), 5.53 (d, J = 5.3 Hz, 1H), 4.85 (d, J = 4.4 Hz, 1H), 4.84 (s, 1H), 3.26 (t, J = 5.1 Hz, 1H), 2.80–2.67 (m, 1H), 2.57–2.48 (m, 1H), 2.05 (s, 3H), 1.40–1.10 (m, 4H), 0.80 (t, J = 7.2 Hz, 3H).**^{13}C NMR (75 MHz, $CDCl_3$)**: δ = 179.1, 171.1, 141.8, 136.5, 129.1, 128.3, 127.5, 126.7, 78.6, 75.8, 74.7, 72.4, 46.6, 28.5, 21.0, 19.8, 13.6. **HRMS *m/z***: $[M+H]^+$ calculated for $C_{19}H_{26}NO_5^+$348,1805, found 348.1798.

CCDC-2210834 contains the supplementary crystallographic data for this paper (compound **11**). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

Acknowledgements

The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for financial support (2022.08559.PTDC, UIDB/04138/2020, UIDP/04138/2020, SFRH/BD/120119/2016, SFRH/BD/148211/2019 and 2021.06598.BD). The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951996. The NMR spectrometers are part of the National NMR Network (PT NMR) are partially supported by Infrastructure Project No. 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

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