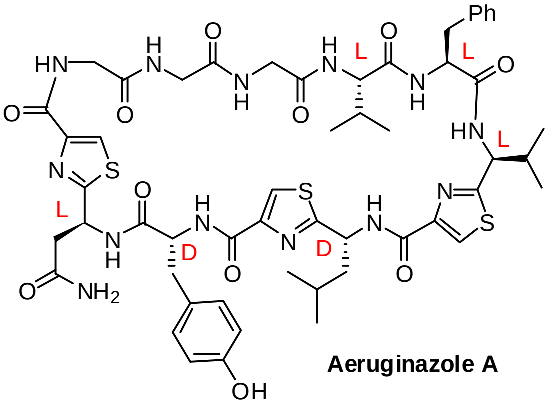
Total synthesis of Aeruginazole A

Paolo Bruno,*[†,¥] Stella Peña,[†,#] Xavier Just-Baringo,[†,¥] Fernando Albericio[†,‡,¥] and Mercedes Álvarez*[†,§,¥]

Barcelona Science Park, Carrer Baldiri Reixac 10, 08028 Barcelona, Spain
mercedes.alvarez@irbbarcelona.org

Received Date (will be automatically inserted after manuscript is accepted)

ABSTRACT



Aeruginazole A

Herein is reported the first total synthesis of Aeruginazole A, prepared via a convergent strategy that involved both solid-phase peptide synthesis and solution phase chemistry and that enabled conservation of the stereochemistry of the intermediates.

Aeruginazole A is a macrocyclic dodecapeptide that was recently isolated from the cyanobacterium *Microcystis* sp. strain (IL-323) and that exhibits inhibitory activity towards *Bacillus subtilis*.[1] It is an interesting example of the numerous macrocyclic thiazole-containing compounds that have been isolated from natural sources over the past few decades and subsequently tested for biological activity, and whose extremely varied structures have been the targets of total syntheses.[2]

The diverse combination of structural motifs in Aeruginazole A is interesting: its macrocycle comprises a pentapeptide of L-amino acids (known as the *northern region*), and a group of three thiazole moieties combined with a D-Tyr residue (the *southern region*).

The presence of L- and D-amino acids, and of amino acid-derived moieties, in the compound is interesting, as is their location: the L-amino acids are located in the *western* and *eastern regions* only, whereas there are two consecutive D-amino acids in the southern region. The stereochemistry of the target molecule is therefore an important feature and a critical synthetic challenge. Intrigued by its peculiar structure and seeking to further explore its biological activity, we decided to undertake the total synthesis of Aeruginazole A.

Work began with the retrosynthetic analysis represented in Figure 1: in addition to the *northern pentapeptide* **1**, tyrosine[3] **5** and the optically active thiazole-building blocks **2-4** were identified through disconnections of the southern region of Aeruginazole A (Figure 1).

The planned route to the stereodefined thiazole building blocks **2-4** was to subject precursor thioamides to Hantzsch thiazole synthesis. These precursors were readily accessed from Boc-L-Asp(OBzl)-OH, Boc-L-Val-OH and Boc-D-Leu-OH. Commercially available *N*-protected amino acids were converted into the corresponding primary amides **6a**-**8a** (Scheme 1) through activation and subsequent treatment with concentrated aqueous ammonia.

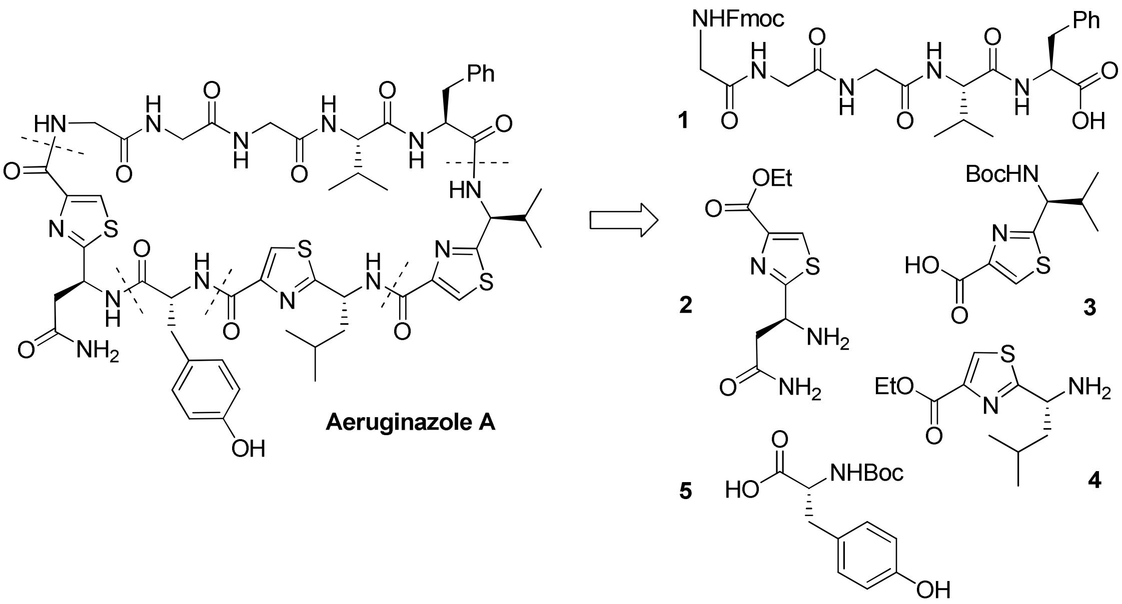


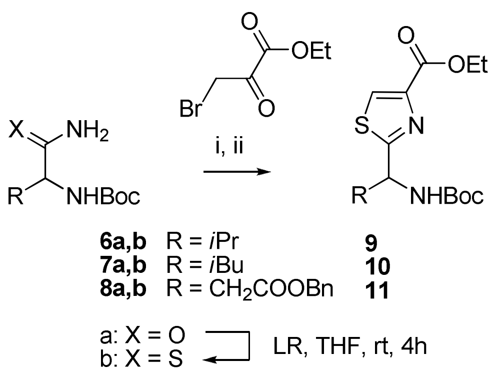
Figure 1. Structure of Aeruginazole A and retrosynthetic analysis

The Leu was activated for nucleophilic substitution through transformation into the corresponding methyl ester.[4] Unfortunately, this easy protocol gave poor yields with Val and was incompatible with the benzyl ester group of protected Asp. Alternatively, these residues were activated by treatment with 2,2,2-trichloroethyl chloroformate. Each of the amides **6a**-**8a** was then converted into its corresponding thioamide **6b**-**8b**, respectively, using Lawesson's reagent (LR).[5]

The three thioamides were then subjected to Hantzsch thiazole syntheses, a critical point in the total synthesis, given the need to preserve the stereochemical information present in the thioamides themselves. First, classical conditions for this reaction were tested: the thioamides were treated with ethyl bromopyruvate and pyridine in refluxing ethanol.[6] Optical purity was checked in the case of the known compound Val-thiazole **9** (Scheme 1) by comparison of its rotatory power with published data.[7]

However, racemization of Leu-thiazole **10** and Asp-thiazole **11** was detected further in the synthetic sequence: NMR spectra revealed formation of diastereomeric mixtures of products when **10** and **11** were converted into the more advanced synthetic intermediates **13** and **14**, respectively (Scheme 3), each of which bears two stereocenters.[8] Once a chiral HPLC analysis method for all three Hantzsch synthesis products had been established, racemization was definitively confirmed (see Experimental Procedures for details). Switching to Merritt and Bagley's protocol[9] (Scheme 1) for Hantzsch synthesis of stereodefined thiazoles provided the building blocks **9**-**11** in 75-98% yields with conservation of optical purity (ee ranging from 91 to 94%). It should be noted that special care had to be paid to the dehydration step (Scheme 1, step ii). Lower-than-expected ee's were observed when the reaction temperature was increased before the dehydration was complete.

Scheme 1. Synthesis of thiazole moieties[a]

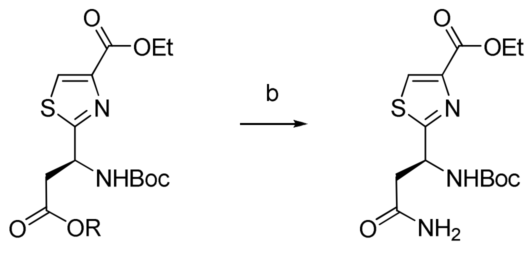


[a]Reagents and conditions: (i) ethyl bromopyruvate, $KHCO_3$, DME, -25 °C to rt, 24 h; (ii) TFAA, 2,6-lutidine, DME, -25 °C, 3 to 4 h

Therefore, termination of the step at -25 °C had to be carefully confirmed before the work up. Furthermore, the previously published work-up procedures had to be slightly modified to enable direct access to the desired compounds.[10]

Once the Hantzsch products **9**-**11** were obtained in suitable optical purity, Asp-thiazole **11** was converted into the desired building block **12** (Scheme 2).

Scheme 2. Synthesis of Asn-thiazole **12**[a]



[a]Reagents and conditions: (a) H_2, Pd/C, *i*PrOH, rt, 24 h, 93%; (b) PyBOP, *N*,*N*-diisopropylethylamine (DIEA), NH_4HCO_3, THF, DMF, 0 °C, 3 h, 68%

The benzyl ester protecting group was cleanly removed by hydrogenolysis; the reaction was first attempted in MeOH but, unexpectedly, partial conversion of the acid to the corresponding methyl ester was observed (from trace amounts up to 53%). This problem was solved by running the reaction in *i*PrOH, which is more sterically hindered. Acid **11a** was then converted into the corresponding amide **12**. The conditions for this transformation had to be extensively investigated, since the initially tested activation strategies and NH_3 sources gave low yields. Actually, activation as mixed anhydrides using either Boc_2O[11] or 2,2,2-trichloroethyl chloroformate, and use of alternative NH_3 sources (conc. aq. NH_3 or NH_4Cl) never gave yields superior to 55%. However, better yields (68%) were obtained when benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)[12] was used as activating agent and ammonium bicarbonate was used as NH_3 source.

The building blocks **9**, **10** and **12** were then deprotected. The ethyl ester of **9** was hydrolyzed with 2N LiOH to give compound **3**; the Boc-protecting groups from **12** and **10** were removed with 4M HCl to afford their corresponding hydrochloride salts **2·HCl** and **4·HCl**, respectively (Scheme 3). At this stage, all the building blocks required for the southern peptide had been synthesized; the preparation of northern peptide was therefore addressed.

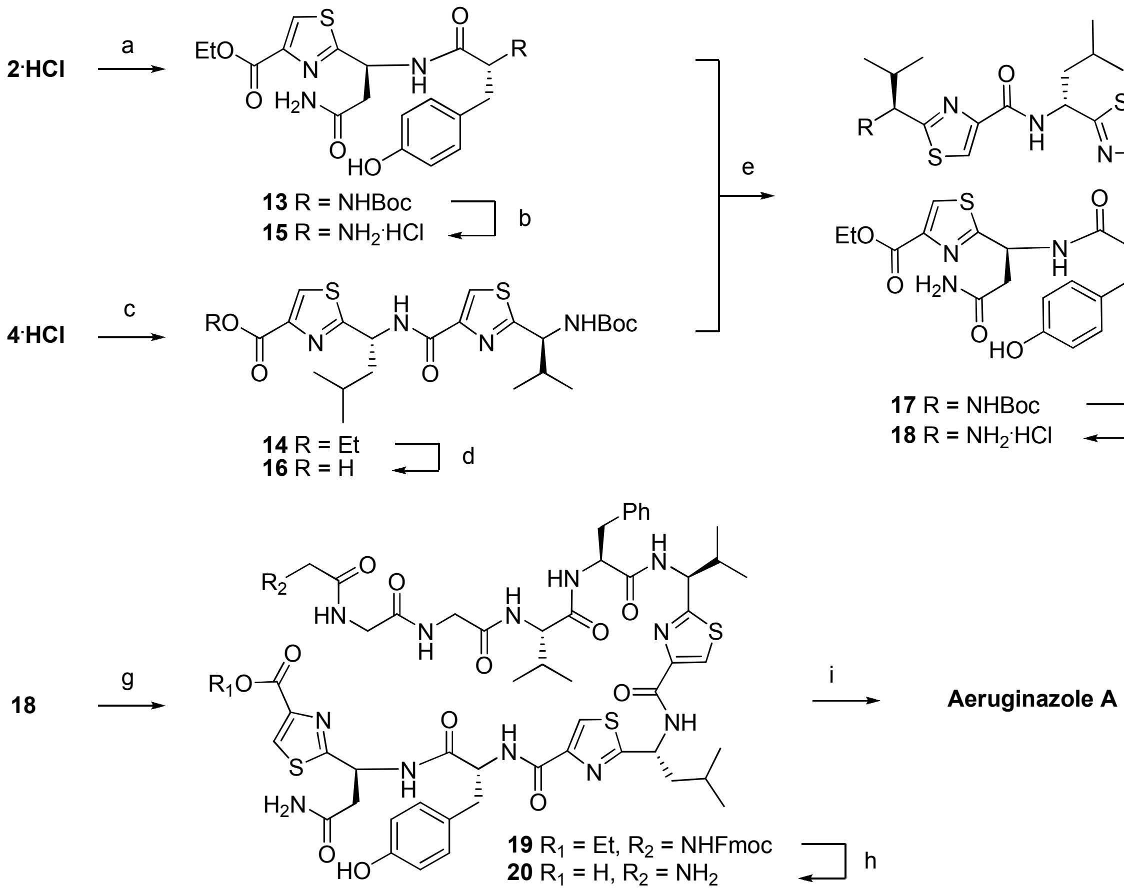
Fmoc-protected pentapeptide **1** was smoothly synthesized by solid-phase peptide synthesis (SPPS) on 2-chlorotritylchloride resin, first manually, through activation with diisopropyl carbodiimide and 1-hydroxybenzotriazole (HOBt), then by adapting the procedure to automated microwave-assisted synthesis using *O*-(benzotriazol-1-yl)-*N*,*N*,*N′*,*N′*-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent.

Since the building blocks **1**-**4** (Figure 1) had been prepared, condensation of the individual synthetic intermediates was then undertaken. The coupling pattern (Scheme 3) was chosen according to the highest possible degree of convergence: Asn-thiazole **2·HCl** was coupled to Boc-D-Tyr-OH, giving rise to building block **13** (91% yield), whereas D-Leu-thiazole **4·HCl** was condensed with Val-thiazole **3** to form building block **14** (79% yield). Both couplings proceeded uneventfully (3 h at 0 °C) through activation by PyBOP.

The amine **15** was then coupled to the acid **16** to form the tris-thiazole peptide **17**. Interestingly, the yield of this reaction was strongly dictated by the ratio of amine to acid: whereas an excess (1.2 equiv) of acid **16** gave a disappointingly low yield (58%), an excess (1.6 equiv) of amine hydrochloride **15** allowed to increase the yield to 92%. The Boc-protecting group was then removed from compound **17** (Scheme 3) to afford the southern peptide **18**, which was directly coupled to northern peptide **1**. The protocol (PyBOP in THF) used in all previous couplings was again tested, but proved unsatisfactory in this case (yields of roughly 10%). Observed solubility issues, as well as a desire to minimize the amount of DIEA used, in consideration of the presence of the Fmoc group, were therefore addressed by switching to a different system, EDCI/HOBt in CH_2Cl_2/DMF, which gave a gratifying yield of 79%. At this stage, the entire linear skeleton of Aeruginazole A had been prepared and thiazole dodecapeptide **19** (Scheme 3) was ready for final hydrolysis of its ethyl ester group, cleavage of its Fmoc group and subsequent macrocyclization. Thus, compound **19** was treated with LiOH in THF/H_2O in a one-pot removal of the protecting groups on both ends of the peptide to give the acid **20**. Macrocyclization of crude **20** was then performed in high dilution conditions (2.5 mM in DMF) by activation with PyBOP and HOAt, affording the desired product in a 24% yield over the two steps of deprotection and cyclization .

The spectroscopic data for this product fully agreed with those for a sample of natural product, thereby enabling confirmation of the structure proposed for Aeruginazole A by Raveh and Carmeli.[1] In summary, Aeruginazole A was obtained in an overall yield of 4.3% through a convergent synthesis combining solution- and solid-phase procedures. Special care was paid to conserve the integrity of the stereocenters in the intermediates, via strict control of Hantzsch thiazole syntheses and through use of various peptide-coupling reagents.

Scheme 3. Total synthesis of Aeruginazole A[a]



[a]Reagents and conditions: (a) **5**, PyBOP, DIEA, THF, 0 °C, 3 h, 91%; (b) 2M HCl, 1,4-dioxane, rt, 2 h, 95%; (c) **3**, PyBOP, DIEA, THF, 0 °C, 3h, 79%; (d) 2N LiOH, THF, rt, 48 h, 97%; (e) PyBOP, DIEA, THF, 0 °C, 3 h, 92%; (f) 2M HCl, 1,4-dioxane, MeOH, rt, 3 h, quant.; (g) **1**, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI), HOBt, DIEA, CH_2Cl_2, DMF, rt, 5 h, 79%; (h) 2N LiOH, THF, H_2O, rt, 4 h; (i) PyBOP, 1-hydroxy-7-azabenzotriazole (HOAt), DIEA, DMF, 0 °C to rt, 60 h, 24% from **19**

Acknowledgment This work was funded by CICYT (Grant CTQ 2009-07758), *Generalitat de Catalunya* (Grant 2009SGR 1024), Barcelona Science Park, the Spanish Ministry of Science and Innovation (MICINN-PTQ 2009), CSIC (UdelaR, Uruguay) and the Chemistry Faculty (UdelaR, Uruguay). We also gratefully thank Prof. Carmeli[13] for precious advice and for sending us original NMR spectra as well as an authentic sample of Aeruginazole A.

Supporting Information Available Experimental procedures and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

† Institute for Research in Biomedicine, Barcelona Science Park, Carrer Baldiri Reixac 10, 08028 Barcelona, Spain

¥ CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine

Current address: Cátedra de Química Farmacéutica (DQO), Facultad de Química, UdelaR, Montevideo, Uruguay

‡ Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain

§ Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain

1. Raveh, A.; Carmeli, S. *Org. Lett.* **2010**, *12,* 3536.

2. For recent reviews on thiazole-containing natural products, see: (a) Davyt, D.; Serra, G. *Mar.* Drugs **2010**, *8,* 2755. (b) Jin, Z. *Nat. Prod. Rep.* **2009**, *26,* 382.

3. The absence of a protecting group on the Tyr phenol (for which a *t*Bu group is normally used), enabled use of Boc for temporary protection of the α-amino function in the southern region, thereby facilitating the synthesis.

4. Xu, Z.; DiCesare, J. C.; Baures, P. W. *J. Comb. Chem.* **2010**, *12,* 248.

5. Müller, H. M.; Delgado, O.; Bach, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 4771.

6. Rzasa, R. M.; Kaller, M. R.; Liu, G.; Magal, E.; Nguyen, T. T.; Osslund, T. D.; Powers, D.; Santora, V. J.; Viswanadhan, V. N.; Wang, H.-L.; Xiong, X.; Zhonga, W.; Norman, M. H. *Bioorg. Med. Chem.* **2007**, *15*, 6574.

7. Downing, S. V.; Aguilar, E.; Meyers, A. I. *J. Org. Chem.* **1999**, *64*, 826.

8. ^{1}H-NMR spectra of compounds **13** and **14** were analyzed: singlets falling in the region from δ 8.00 to 8.15 ppm, relative to the protons in position 5 of each thiazole ring, were used as diagnostic signals.

Observation of a second set of these signals revealed that substrates of low optical purity were coupled, thus forming mixtures of diastereomeric products.

9. Merritt, E. A; Bagley, M. C. *Synthesis* **2007**, *22*, 3535.

10. The original paper described direct concentration of the reaction mixtures with consequent trifluoroacetylation of Boc-protected amino groups: an additional transformation was therefore required to remove the trifluoroacetyl groups and provide access to the desired products. This problem was solved by quenching excess TFAA with saturated aqueous $NaHCO_3$, providing thiazoles **9-11** directly.

11. (a) Pozdnev, V. F. *Tetrahedron Lett.* **1995**, *36*, 7115. (b) Ardá, A.; Soengas, R. G.; M. Nieto, M. I.; Jiménez, C.; Rodríguez, J. *Org. Lett.*, **2008**, *10*, 2175. (c) Trotter, N. S.; Brimble, M. A.; Harris, P. W. R.; Callis, D. J.; Sieg, F. *Bioorg. Med. Chem.* **2005**, *13*, 501.

12. (a) Kahlon, D. K.; Lansdell, T. A.; Fisk, J. S.; Hupp, C. D.; Friebe, T. L.; Hovde, S.; Jones, A. D.; Dyer, R. D.; Henry, R. W.; Tepe, J. J. *J. Med. Chem.* **2009,** *52*, 1302. (b) Arienti, K. L.; Brunmark, A.; Axe, F. U.; McClure, K.; Lee, A.; Blevitt, J.; Neff, D. K.; Huang, L.; Crawford, S.; Pandit, C. R.; Lars Karlsson, L.; Breitenbucher, J. G. *J. Med. Chem.* **2005**, *48*, 1873.

13. Raymond and Beverly Sackler School of Chemistry and Faculty of Exact Sciences, Tel-Aviv University, Ramat Aviv, Tel-Aviv 69978, Israel.