Total Synthesis of Skyllamycins A-C

Andrew M. Giltrap,[a] F. P. Jake Haeckl,[b] Kenji L. Kurita,[b] Roger G. Linington[b] and Richard J. Payne[a]*

Dedicated to Professor Chris Abell on the occasion of his 60th Birthday

Abstract: The skyllamycins are a family of highly functionalized non-ribosomal cyclic depsipeptide natural products which contain the extremely rare α-OH-glycine functionality. Herein we report the first total synthesis of skyllamycins A-C, together with the biofilm inhibitory activity of the natural products. Linear peptide precursors for each natural product were prepared through an efficient solid-phase route incorporating a number of synthetic modified amino acids. A novel macrocyclization step between a C-terminal amide and a N-terminal glyoxylamide moiety served as a key transformation to install the unique α-OH-glycine unit and generate the natural products in the final step of the synthesis.

Skyllamycins A (**1**), B (**2**), and C (**3**) are a family of non-ribosomal cyclic depsipeptide natural products isolated from *Steptomyces sp.*[1] (Figure 1). They contain a number of unusual structural features: three β-OH amino acids (β-OH-Phe, β-OH-*O*-Me-Tyr, β-OH-Leu) (blue), an N-terminal cinnamoyl residue (red), a β-Me-aspartic acid (β-Me-Asp) (green) and the extremely rare α-OH-Gly residue (purple). The biosynthetic origin of these modifications has been recently investigated.[1b] A single P450 monoxygenase ($P450_{sky}$) was found to be responsible for catalyzing the β-hydroxylation of Phe, Leu and *O*-Me-Tyr, producing the (3*S*)-diastereomer in each case,[2] however the exact timing at which the α-OH-Gly residue is installed remains unknown. Indeed, α-OH-Gly residues are extremely rare and to date this unusual moiety has only been found in one other natural product, the structurally simpler linear peptide anti-tumour agent spergualin.[3]

The stereochemistry of the amino acids present in **1**-**3** was not assigned during the initial isolation; however further work by Süssmuth and co-workers[4] confirmed the absolute configuration of the amino acids *via* a combination of chiral HPLC/GC and Marfey's analysis. Due to the instability of the α-OH-Gly residue, the configuration could not be confirmed experimentally. Instead, by estimating the distances between protons using NOESY measurements, followed by molecular dynamics simulations, the authors proposed that the α-OH-Gly residue was (*S*)-configured. These calculations also predicted the presence of a strong intramolecular hydrogen bond network when the α-OH-Gly residue was in the (*S*)-configuration.

[a] Andrew Giltrap and Prof. Richard J. Payne
School of Chemistry
The University of Sydney
Sydney, NSW 2006, AUSTRALIA
E-mail: richard.payne@sydney.edu.au
[b] F. P. Jake Haeckl, Dr Kenji L. Kurita and Prof. Roger L. Linington
Department of Chemistry
Simon Fraser University
Burnaby, British Columbia BC V5A 1S6, Canada

Supporting information for this article is given via a link at the end of the document.

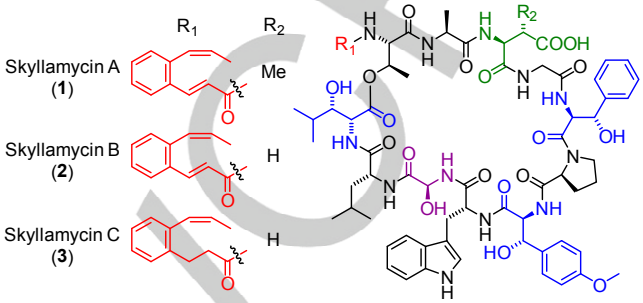


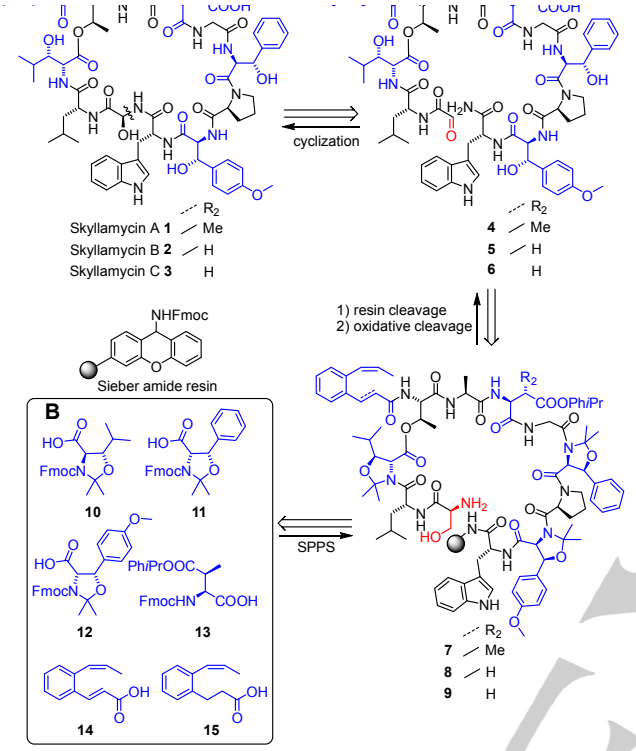
Figure 1. Structure of skyllamycins A-C (**1**-**3**).

The skyllamycins also possess interesting biological activity. They were initially discovered in the search for novel agents that inhibit the platelet-derived growth factor (PDGF) signaling pathway. We recently re-isolated them in our search for inhibitors of *Pseudomonas aeruginosa* biolfilms.[1c] Traditional antibiotics target bacteria in the platonic state, however the formation of biofilms allow bacteria to evade their action.[5] As such, agents which inhibit biofilm formation or clear pre-attached biofilms, a major contributor to the persistence of nosocomial infections, represent a potentially useful therapeutic strategy. To our knowledge, the skyllamycins are the first known class of cyclic peptide biofilm disruptors. Given their highly unusual structural features, together with their important anti-biofilm activity, we sought to develop a concise total synthesis of skyllamycins A-C (**1**-**3**) which is described herein which, to date, have not succumbed to total synthesis.

Retrosynthetically, we envisioned a novel macrocyclization step to generate the α-OH-Gly residue that would necessitate preparation of modified linear peptides **4**-**6** containing a C-terminal amide and a N-terminal glyoxylamide functionality (Scheme 1A). We reasoned that, due to the inherent instability of the α-OH-Gly in linear peptides,[6] formation in the final step would reduce the likelihood of decomposition during synthesis. Indeed, the stability of this residue in the natural products is proposed to be due to a hydrogen-bonding network in the mature structure.[1b] Furthermore, the presence of this intramolecular hydrogen-bonding network during the cyclization reaction could potentially induce α-OH-Gly formation with the desired (*S*)-stereochemistry. Glyoxylamides **4**-**6** in turn could be accessed from resin-bound **7**-**9** by resin cleavage followed by oxidative cleavage of the N-terminal serine residue.[7] We envisioned that resin-bound peptides **7**-**9** could be prepared *via* Fmoc-solid-phase peptide synthesis (SPPS) from Sieber amide resin which possesses a linker that can be cleaved under mild acidic conditions (1% TFA in CH_2Cl_2).[8]

Before the assembly of skyllamycins A-C could begin, synthesis of the unusual amino acids present in the natural products was required (Scheme 1B). The suitably protected β-OH-Leu **10**, β-OH-Phe **11**, β-OH-*O*-Me-Tyr **12** were synthesized utilizing a unified strategy, namely organometallic addition to Garner's aldehyde[9] followed by deprotection, oxidation and protecting group manipulations[10] (see Supporting Information for synthetic details). The β-OH groups were protected as an oxazolidine, based on the pseudo-proline moiety commonly

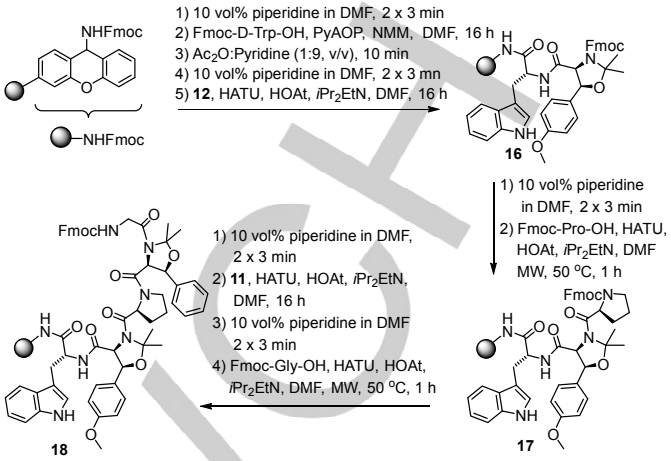
used for serine and threonine residues in SPPS[11] to improve the quality of the linear peptide synthesized through the induction of turns in the peptide backbone. Protected β-Me-Asp **13** was synthesized by adapting a procedure from Goodman *et al.*[12] Finally, cinnamic acids **14** and **15** were synthesized *via* Sonogashira cross-coupling followed by Lindlar reduction.[13]



Scheme 1. A) Retrosynthetic analysis of skyllamycins A-C (**1**-**3**). **B)** Synthetic building blocks **10**-**15** required for the total synthesis of **1**-**3**.

Our initial synthetic efforts toward linear peptide precursors **7**-**9** employed strongly acidic conditions for side-chain deprotection which caused decomposition of the β-OH-*O*-Me-Tyr residue *via* deoxygenation and dehydration pathways (an observation also made in the synthesis of Callipeltin B by Lipton and co-workers).[14] This prevented the use of the standard protecting groups used in Fmoc-SPPS. As such, the more exotic phenyl isopropyl group (Ph*i*Pr) was used for the protection of the side chain carboxylate of Asp/β-Me-Asp that can be cleaved under mildly acidic conditions,[15] while the side chain indole of the D-Trp residue was carried through the SPPS unprotected.

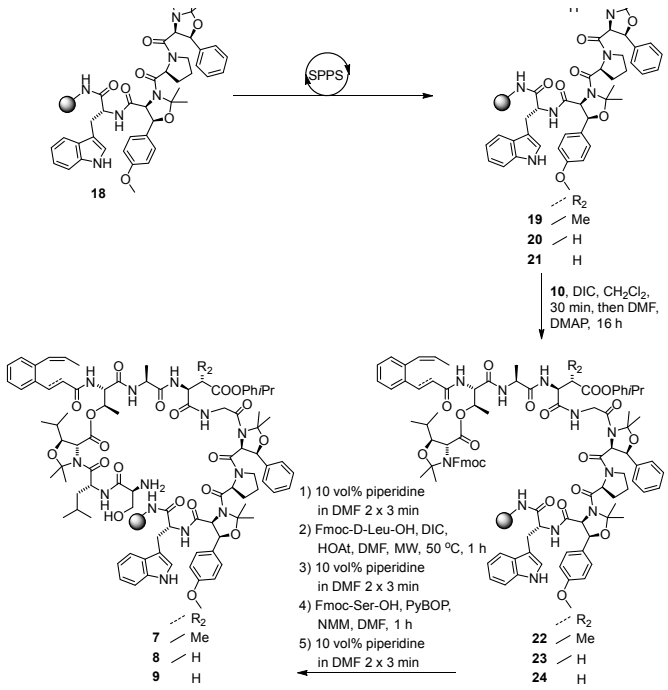
To begin the synthesis, Fmoc-D-Trp-OH was loaded onto Sieber amide resin using (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) and *N*-methylmorpholine (NMM) (Scheme 2). Next, oxazolidine-protected Fmoc- β-OH-*O*-Me-Tyr-OH (**12**) was coupled for 16 h using 1-[*bis*(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt) and *i*Pr$_2$NEt to yield resin-bound dipeptide **16**. Due to the hindered nature of the oxazolidine-protected N-terminus, Fmoc-Pro-OH was coupled under optimized conditions, namely HATU, HOAt and *i*Pr$_2$NEt with microwave irradiation at 50 °C for 1 hour to afford **17**. Protected Fmoc-β-OH-Phe-OH (**11**) and Fmoc-Gly-OH were subsequently coupled, the latter under microwave conditions, to yield the resin-bound pentapeptide **18**.



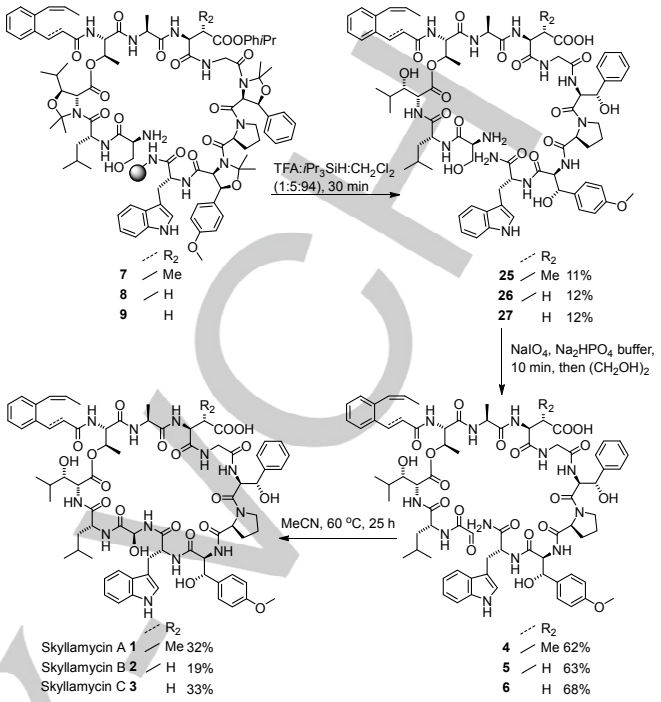
Scheme 2. Synthesis of resin-bound intermediate **18.**

Resin-bound **18** served as the key intermediate for the synthesis of the three natural products (**1**-**3**). In the case of skyllamycin A (**1**), Fmoc-β-Me-Asp(Ph*i*Pr)-OH (**13**) was coupled to resin-bound **18** for 16 h, employing HATU coupling conditions (Scheme 3). The synthesis of skyllamycins B (**2**) and C (**3**) was continued by the coupling of Fmoc-Asp(Ph*i*Pr)-OH. Iterative Fmoc-SPPS utilizing benzotriazol-1-yl-oxytripyrrolidino-phosphonium hexafluorophosphate (PyBOP) to couple Fmoc-Ala-OH and side chain unprotected Fmoc-Thr-OH, followed by HATU-mediated coupling of cinnamic acid **14** or **15** afforded resin-bound peptides **19**-**21**.

Next, the key on-resin esterification step was carried out utilizing oxazolidine-protected Fmoc-β-OH-Leu-OH **10**, *N,N'*-di*iso*propylcarbodiimide (DIC) and catalytic *N,N*-dimethylaminopyridine (DMAP) affording resin-bound **22**-**24**. Importantly, we could not detect any epimerization of the β-OH-Leu moiety under the DMAP-catalyzed esterification conditions. With the key ester bond now in place, Fmoc-D-Leu-OH was effectively coupled to resin-bound peptides **23** and **24** as described previously, using HATU, HOAt and *i*Pr$_2$NEt at 50 °C for 1 hour under microwave heating. Unfortunately, when these conditions were applied to resin-bound intermediate **22** containing the β-Me-Asp residue, a significant amount of by-product with a mass 18 Da less than the product was observed. This was likely owing to aspartimide formation[16] promoted by a conformational change in the peptide backbone induced by the β-Me group. Gratifyingly, this side-reaction could be suppressed by excluding an additional base from the coupling reaction, instead utilizing DIC and HOAt at 50 °C with microwave heating. These optimized conditions were subsequently employed, followed by elongation with side chain unprotected Fmoc-Ser-OH to afford resin-bound peptides **7**-**9**.



Scheme 3. Synthesis of resin-bound intermediates **7**-**9**.



Scheme 4. Total Synthesis of skyllamycins A-C (**1**-**3**).

With the fully assembled linear resin-bound intermediates **7**-**9** in hand, the next step involved cleavage from resin. To this end, resin-bound **7**-**9** were treated with 1% TFA in CH_2Cl_2 with 5% *i*Pr_3SiH as a cation scavenger, affording the desired linear peptides **25**-**27** in 11-12% yield after HPLC purification. Importantly, these mildly acidic conditions effected removal of the Ph*i*Pr group and cleavage of the pseudo-proline isopropylidene moieties.

Having generated linear peptides **25**-**27**, the key oxidative cleavage step was next attempted on the N-terminal Ser residue to generate the requisite glyoxylamide moiety. To this end, a solution of $NaIO_4$ (2 eq.) was added to **25**-**27** in a 1:1 v/v mixture of Na_2HPO_4 buffer (pH = 9) and MeCN for 10 min before being quenched with ethylene glycol, acidified and immediately purified by HPLC. Pleasingly, this yielded target glyoxylamides **4**-**6** in 62-68% yield after HPLC purification. The identity of the glyoxylamide was confirmed by the presence of the corresponding hydrate $[M+H_3O]^+$ ion in the MS[7a] (see Supporting Information).

With the glyoxylamides **4**-**6** in hand, the critical cyclization with concomitant formation of the α-OH-Gly residue was attempted on skyllamycin B precursor **5**. Initially glyoxylamide **5** was reacted in a solution of 1% TFA in MeCN for 20 h and the reaction was monitored by HPLC-MS. Gratifyingly, two new peaks were observed during HPLC analysis, both with the desired mass of skyllamycin B (**2**). Unfortunately, when the HPLC purified products were analyzed by ^{1}H-NMR spectroscopy neither compound was a good match for the authentic natural product. While the identity of the products of the cyclization was not determined, we hypothesize that these products are cyclic hemiacetals formed *via* the attack of one of the side chain hydroxyls onto the glyoxylamide.

Interestingly, the next conditions trialed, namely incubation of glyoxylamide **5** in MeCN at 60 °C at a final concentration of 1 mM for 25 h, resulted in the consumption of starting material, and the formation of a new peak, again with the mass of the desired natural product **2** but with a different retention time to those generated under the acidic conditions above. This new product was isolated by HPLC in 42% yield. Gratifyingly, whilst the isolated material was a 1:1 mixture of two compounds, significant overlap was observed between one set of signals in the ^{1}H-NMR and the spectrum of the isolated natural product. The synthetic mixture was next compared to an authentic sample of skyllamycin B (**2**) *via* UPLC-MS co-injection experiments, which further supported the presence of the natural product. Finally, this mixture was separated by an optimized HPLC protocol to afford pure synthetic skyllamycin B (**2**) in 19% yield from the cyclization reaction that possessed almost identical NMR spectra to the isolated natural product (see Supporting Information).

With the cyclization methodology validated on skyllamycin B (**2**), glyoxylamides **4** and **6** were subjected to the same conditions, namely incubation in MeCN at 60 °C for 25 h at a concentration of 1 mM. Analysis of the crude reactions revealed the consumption of starting material and formation of new peaks with the desired mass of the natural products. After HPLC purification, synthetic skyllamycins A (**1**) and C (**3**) were isolated in 32% and 33% yield, respectively from the cyclization reaction. The retention time of synthetic **1** and **3** was shown to be identical to the isolated natural products *via* UPLC-MS co-injection studies (see Supporting Information). In addition, comprehensive NMR comparisons between synthetic and isolated skyllamycins A (**1**), B (**2**) and C (**3**) showed very strong overlap suggesting that the synthetic materials were identical to the authentic isolated natural products (see Supporting Information). Furthermore, highly analogous ORD spectra

between the synthetic and isolated skyllamycins A-C suggested that they were the same enantiomer (see Supporting Information). Finally, the synthetic natural products were analyzed in a *P. aeruginosa* biofilm inhibition assay alongside the authentic natural products. Pleasingly, the synthetic and isolated products **1**-**3** showed comparable biofilm inhibition activity, further confirming the identity of the synthetic natural products (see Supporting Information).

In conclusion, we have successfully completed the first total synthesis of skyllamycins A-C (**1**-**3**). A key final macrocyclization step with concomitant formation of the unusual α-OH-Gly moiety was used to construct the natural products, which were identical in all respects to isolated skyllamycins A-C. This work lays the foundation for further studies into the biosynthesis of these natural products, the generation of synthetic skyllamycin analogues, as well as the interrogation of the mechanism of their biofilm inhibitory activity. Studies toward this end will be reported in due course.

Acknowledgements

We thank Dr. Ian Luck and Dr. Nick Proschogo (University of Sydney) for NMR spectroscopy and mass spectrometry technical support, respectively, and Walter Bray (UC, Santa Cruz) for support with the biofilm screening. We thank the Australian Postgraduate Award and John A. Lamberton Scholarship for PhD funding (A.M.G), ARC Future Fellowship to R.J.P (FT130100150), and NSERC Discovery support to R.G.L. (RGPIN-2016-03962).

Keywords: solid-phase synthesis • natural products • biofilms • peptides • cyclic peptides

[1] a) S. Toki, T. Agatsuma, K. Ochiai, Y. Saitoh, K. Ando, S. Nakanishi, N. A. Lokker, N. A. Giese, Y. Matsuda, *J. Antibiot.* **2001**, *54*, 405-414; b) S. Pohle, C. Appelt, M. Roux, H. P. Fiedler, R. D. Süssmuth, *J. Am. Chem. Soc.* **2011**, *133*, 6194-6205; c) G. Navarro, A. T. Cheng, K. C. Peach, W. M. Bray, V. S. Bernan, F. H. Yildiz, R. G. Linington, *Antimicrob. Agents Chemother.* **2014**, *58*, 1092-1099.

[2] a) S. Uhlmann, R. D. Süssmuth, M. J. Cryle, *ACS Chem. Biol.* **2013**, *8*, 2586-2596; b) K. Haslinger, C. Brieke, S. Uhlmann, L. Sieverling, R. D. Süssmuth, M. J. Cryle, *Angew. Chem. Int. Ed.* **2014**, *53*, 8518-8522.

[3] a) T. Takeuchi, H. Iinuma, S. Kunimoto, T. Masuda, M. Ishizuka, M. Takeuchi, M. Hamada, H. Naganawa, S. Kondo, H. Umezawa, *J. Antibiot.* **1981**, *34*, 1619-1621; b) H. Umezawa, S. Kondo, H. Iinuma, S. Kunimoto, Y. Ikeda, H. Iwasawa, D. Ikeda, T. Takeuchi, *J. Antibiot.* **1981**, *34*, 1622-1624.

[4] V. Schubert, F. Di Meo, P. L. Saaidi, S. Bartoschek, H. P. Fiedler, P. Trouillas, R. D. Süssmuth, *Chem. Eur. J.* **2014**, *20*, 4948-4955.

[5] P. S. Stewart, J. William Costerton, *The Lancet* **2001**, *358*, 135-138.

[6] L. Lebreton, J. Annat, P. Derrepas, P. Dutartre, P. Renaut, *J. Med. Chem.* **1999**, *42*, 277-290.

[7] a) K. F. Geoghegan, J. G. Stroh, *Bioconjugate Chem.* **1992**, *3*, 138-146; b) O. El-Mahdi, O. Melnyk, *Bioconjugate Chem.* **2013**, *24*, 735-765.

[8] P. Sieber, *Tetrahedron Lett.* **1987**, *28*, 2107-2110.

[9] P. Garner, J. M. Park, *J. Org. Chem.* **1987**, *52*, 2361-2364.

[10] a) L. Williams, Z. D. Zhang, F. Shao, P. J. Carroll, M. M. Joullié, *Tetrahedron* **1996**, *52*, 11673-11694; b) N. Okamoto, O. Hara, K. Makino, Y. Hamada, *J. Org. Chem.* **2002**, *67*, 9210-9215; c) A. Nishida, H. Sorimachi, M. Iwaida, M. Matsumizu, T. Kawate, M. Nakagawa, *Synlett* **1998**, 389-390.

[11] T. Wöhr, F. Wahl, A. Nefzi, B. Rohwedder, T. Sato, X. Sun, M. Mutter, *J. Am. Chem. Soc.* **1996**, *118*, 9218-9227.

[12] S. Schabbert, M. D. Pierschbacher, R. H. Mattern, M. Goodman, *Bioorg. Med. Chem.* **2002**, *10*, 3331-3337.

[13] D. Sun, P. Lai, W. Xie, J. Deng, Y. Jiang, *Synth. Commun.* **2007**, *37*, 2989-2994.

[14] R. Krishnamoorthy, L. D. Vazquez-Serrano, J. A. Turk, J. A. Kowalski, A. G. Benson, N. T. Breaux, M. A. Lipton, *J. Am. Chem. Soc.* **2006**, *128*, 15392-15393.

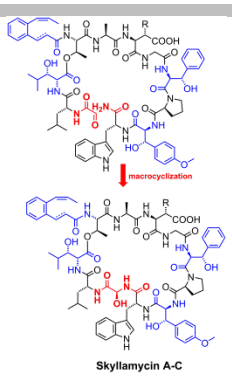
[15] C. Yue, J. Thierry, P. Potier, *Tetrahedron Lett.* **1993**, *34*, 323-326.

[16] R. Subirós-Funosas, A. El-Faham, F. Albericio, *Tetrahedron* **2011**, *67*, 8595-8606.

Entry for the Table of Contents

COMMUNICATION

The total synthesis of the complex cyclic peptide natural products skyllamycin A, B and C is described. The successful syntheses hinged on an unprecedented macrocyclization reaction in the last step that furnished the natural products through the generation of the unusual α-OH-Gly moiety.



*Andrew M. Giltrap, F. P. Jake Haeckl, Kenji L. Kurita, Roger G. Linington and Richard J. Payne**

Page No. – Page No.

Total Synthesis of Skyllamycins A-C