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A facile approach to tryptophan derivatives for the total synthesis of argyrin analogues†

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A facile route has been established for the synthesis of indole-substituted (*S*)-tryptophans from corresponding indoles, which utilizes a chiral auxiliary-facilitated Strecker amino acid synthesis strategy. The chiral auxiliary reagents evaluated were (*S*)-methylbenzylamine and related derivatives. To illustrate the robustness of the method, eight optically pure (*S*)-tryptophan analogues were synthesized, which were subsequently used for the convergent synthesis of a potent antibacterial agent, argyrin A and its analogues.

Tryptophan and its derivatives are key biosynthetic precursors in many nonribosomal peptide antibiotics. These peptidic compounds typically display a wide spectrum of biological activities. Specifically, the argyryns, a family of eight 24-membered macrocyclic peptides isolated from the marine myxobacterium *Archangium gephyra*,^{1,2} have been associated with antibacterial,^{2–4} antiinflammatory² and antiproteasomal^{5,6} activities. All biologically active members of the family contain an unusual (*S*)-4-methoxy-tryptophan residue.^{1–6} Argyrin A and B display potent antibacterial activity against the opportunistic human pathogen *Pseudomonas aeruginosa*,^{1,2,7,8} which is the result of functional inhibition of elongation factor G (EF-G).^{7,8} Utilizing the nomenclature recently introduced by Villar *et al.*,⁹ argyrin B⁷ appeared to engage in a compact binding mode with EF-G, in which ‘peripheral atoms’ and ‘substituent atoms’ make extensive contacts with EF-G. This binding mode reveals intimate polar and nonpolar interactions between EF-G and the 4-methoxy-tryptophan side-chain (substituent atoms) of argyrin B.

While the (*S*)-4-methoxy-tryptophan residue in argyrin A has been identified as crucial for its antiproteasomal activity in human cancer cell lines,⁶ the structure–activity relationships (SARs) of this residue that govern the peptide antibacter-

ial property remain unexplored. Therefore a variety of (*S*)-Trp analogues were prepared in order to investigate the role of this indolyl amino acid residue in antibacterial activity.

Current methods employed for the synthesis of enantiomerically pure (*S*)-Trp analogues rely on either enzymatic or chemical approaches. The enzymatic approach involves a final-step resolution of the racemates using *N*-acylase enzyme, which affords a mixture of the desired product and the unprocessed (*R*)-*N*-acetyl-tryptophan.¹⁰ Chemical approaches in the past have exploited various chiral auxiliaries to access enantiomerically pure (*S*)-Trp analogues. For example, Ma *et al.*¹¹ utilize a Schöllkopf chiral auxiliary whilst Buelow *et al.*⁶ employ a DuanPhos ligand. We herein report our progress towards the development of a flexible and operationally simple synthetic route to a raft of (*S*)-Trp analogues. Our strategy exploits a previously reported asymmetric Strecker synthesis of aliphatic α -aminonitriles that employs chiral auxiliary reagents, such as (*S*)- α -methylbenzylamine, which in turn delivers aliphatic (*S*)-amino acids.¹² The new (*S*)-Trp analogues were then used for the total chemical synthesis of argyrin A and related analogues. In contrast to previously reported solution-based fragment condensation approaches,^{10a,13} we also report for the first time a robust Fmoc/*t*Bu solid-phase peptide synthesis (SPPS) strategy for the stepwise assembly of the octapeptide.

In our appraisal of the α -substituted benzylamine-facilitated Strecker synthesis of new (*S*)-Trp analogues (Scheme 1), several key challenges were addressed, including the stability of intermediates, the availability of chiral auxiliary reagents that are inexpensive and are tolerant to a wide range of substrates, and the stereoselectivity of the condensation reaction.

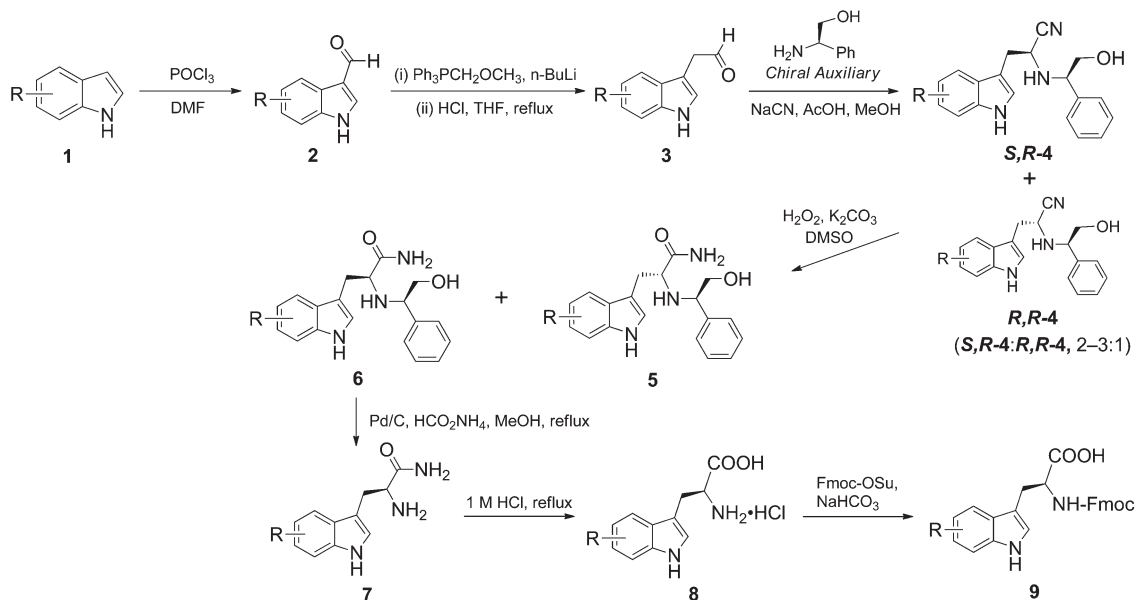
Thus, a variety of commercially available indoles **1** were subjected to Vilsmeier formylation to give indole-3-carbaldehydes **2**, which were then homologated *via* a Wittig reaction to afford the 2-(indol-3-yl)acetaldehydes **3**. In the latter transformation, efficiency of the acid hydrolysis of the enol ether intermediates was found to be dependent on the electronic nature of substituents in the indole ring. Regardless of the position, indoles with an electron-donating substituent (**2e–h**) were more labile to acid-mediated decomposition than

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Scheme 1 Synthesis of tryptophan analogues.

Table 1 Homologation of indole-3-aldehyde derivatives 2 followed by hydrolysis to the 2-(1H-indol-3-yl)acetaldehydes 3

Compound 3	R =	HCl (M)	Reaction time (h)	Crude yield (%)
a	H	0.4	2.5	69
b	5-Br	0.4	2	74
c	5-Cl	0.4	2	74
d	6-F	0.4	2	73
e	7-Et	0.4	1.5	65
f	5-Me	0.4	1.5	60
g	5-MeO	0.1	1.5	55
h	4-MeO	0.1	1.5	47

their counterparts with an electron-withdrawing substituent (2b–d) (Table 1). Moreover, the 2-(indol-3-yl)acetaldehydes 3e–h were typically found to be unstable on storage. Hence, the two-step homologation reaction was carefully monitored by TLC to avoid decomposition and the reaction products were immediately subjected to Strecker condensation to yield the α -aminonitriles 4.

For the crucial three-component Strecker condensation reaction, we started our study by evaluating the effectiveness of the chiral auxiliary agent (*R*)-2-phenylglycinol **10**¹⁴ in comparison to the standard reagent (*S*)- α -methylbenzylamine **11** (Fig. 1).¹² Using 2-(indol-3-yl)acetaldehyde **3a** as the model substrate, initial condensation of **10** with the protonated aldehyde **3a** generates an imine intermediate which in turn is attacked by a cyanide anion to yield the α -aminonitrile **4a**.¹² As anticipated, the nucleophilic nitrile addition occurs at the more favourable, less shielded diastereotopic *re*-face to afford the (*S,R*)-diastereomer **4a** as the predominant product. Similarly, (*S*)- α -methylbenzylamine **11** gave the *re*-addition product, a (*S,S*) variant of **4a**. Both the chiral auxiliary agents **10** and **11** gave the products **4** with a favourable dr 2–3:1, which was

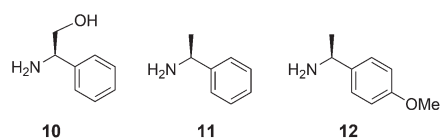


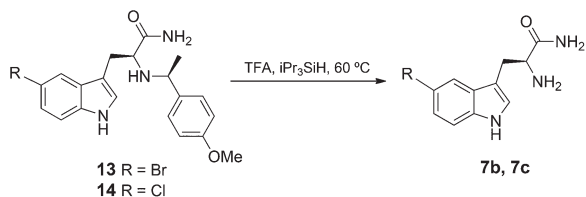
Fig. 1 The chiral auxiliary amine reagents used in the Strecker synthesis strategy.

determined by RP-HPLC analysis; a dr in this range was also observed for all indole variants (a–h) examined in this study.

For the next transformation step, initial attempts to hydrolyse the α -aminonitriles **4** directly to α -amino acids proved to be difficult. Typical acid- or base-catalysed hydrolytic methods required concentrated acid or strong alkali at high temperature,¹⁵ which in our case resulted in an intractable mixture of decomposition products. Hence, we opted for an indirect route, by converting the α -aminonitriles **4** to α -amino amides **5** and **6**, which was achieved by employing 30% aqueous H₂O₂–DMSO–K₂CO₃.^{16,17} The additive DMSO significantly accelerated the reaction under mild basic conditions. In fact, the H₂O₂-enabled oxidation of α -aminonitriles was highly efficient and gave exclusively the α -amino amide diastereomers **5** and **6** in good yields. At this stage, we found that the α -amino amides **5** and **6** were readily and efficiently separated by preparative silica gel flash-chromatography, to deliver the required diastereomerically pure compounds **6**. It is worth noting that, when (*S*)- α -methylbenzylamine **11** was utilized, we were unable to achieve reliable separation of the corresponding α -(methylbenzylamino) amides by column chromatography.

Since the *S*-isomer is the required enantiomer, compounds **6** were used for subsequent investigations. We next established that an unmasking step, *i.e.* the debenzoylation step, followed by mild acid hydrolysis was the most robust procedure to finally furnish the (*S*)-Trp analogues **8**. It has been previously





Scheme 2 The acidolytic removal of *N*-PMB group.

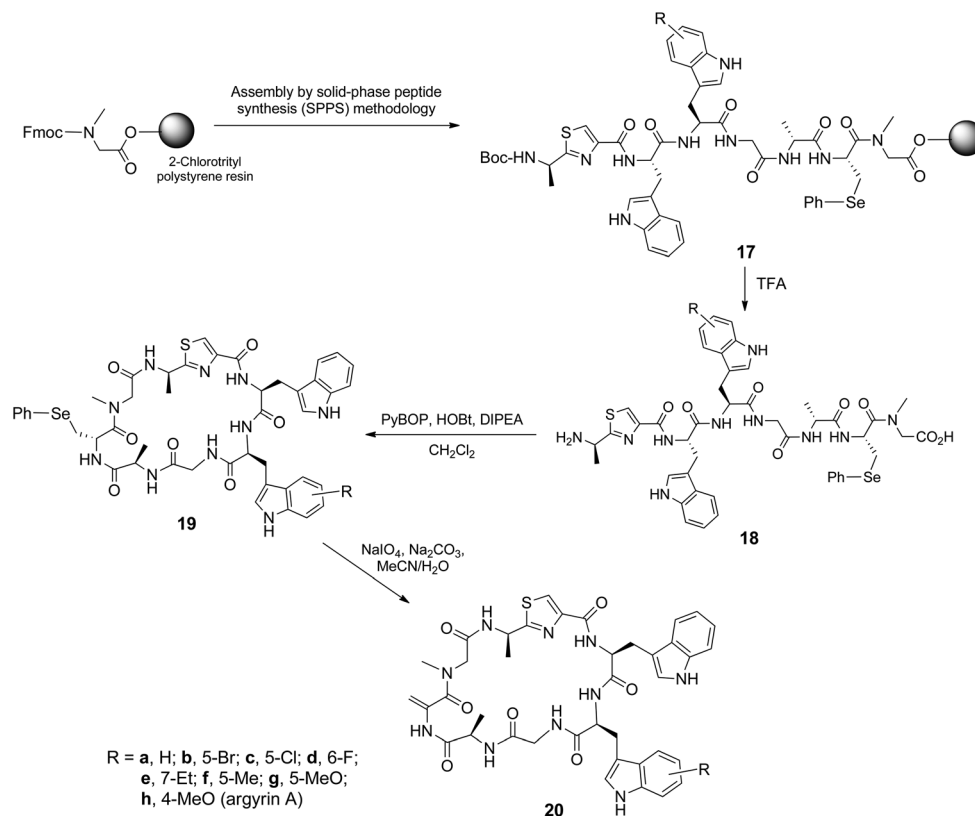
reported that the methyl group in the α -position could prevent *N*-debenzylation under standard hydrogenolysis conditions.¹⁸ Although the use of the more active Pearlman's catalyst Pd(OH)₂/C to remove the *N*- α -methylbenzyl group was considered, it poses a risk of reducing the indole system.^{19–21} To avoid this, we employed catalytic phase transfer hydrogenolysis to promote the *N*-debenzylation of compounds **6**.²² Accordingly, 10% Pd/C in the presence of excess ammonium formate in MeOH,²³ was used to afford specifically **7a** and **7d–h**.

However, hydrogenolysis of the halogen-substituted compounds **6b** and **6c** led to concomitant dehalogenation, resulting in **7a** as the only isolated product. Selective hydrogenolytic debenzylation in the presence of bromo- and chloro-substituted phenyls remain poorly explored. Li *et al.* reported the use of chloride salt in the selective hydrogenolysis of *O*-benzyl groups in the presence of aryl chloride.²⁴ Unfortunately, using similar conditions, we observed dehalogenation of our substrates **6b** and **6c**.

To overcome the above problem, we investigated the use of an alternative chiral auxiliary reagent, 4-methoxy- α -methylbenzylamine (PMB) **12**, to generate the α -amino amides **13** and **14**. Various oxidative, reductive and acidolytic methods could subsequently be used to remove the PMB group.²⁵ Our initial attempts of oxidative removal using CAN or DDQ²⁶ were unsuccessful. Gratifyingly, treatment of **13** and **14** with TFA under reflux enabled efficient acidolytic removal of PMB to yield **7b** and **7c**, respectively (Scheme 2).

The unmasked α -amino amides **7a–h** were subsequently subjected to mild hydrolysis using 1 M aqueous HCl under reflux condition for 5 h. This afforded the α -amino acids **8** in good-to-excellent yields (>80%). Since these new tryptophan analogues are required for peptide synthesis, without further purification, compounds **8** were Fmoc-protected using standard procedures to afford **9**. In addition to using ¹H NMR to establish purity, their absolute configuration was established. Specifically, the observed [α]_D²⁴ –29 and –23 for **9a** and **9g**, respectively, are comparable to the values reported in the literature, [α]_D²⁵ –29.5 (*c* 1, DMF)²⁷ and –24.5 (*c* 1, MeOH).^{10b} The outlined synthetic routes therefore delivered a raft of enantiomerically pure (*S*)-Fmoc-tryptophan analogues.

The Fmoc-protected amino acids **9** were then used for the total chemical synthesis of argyrin A and analogues thereof. This entailed stepwise assembly of the peptide sequence using Fmoc/*t*Bu solid-phase peptide synthesis (SPPS) followed by acidolytic (TFA–CH₂Cl₂–H₂O–iPr₃SiH) release of the linear



Scheme 3 Fmoc/*t*Bu solid-phase synthesis followed by macrocyclization to afford argyrin A and its analogues.



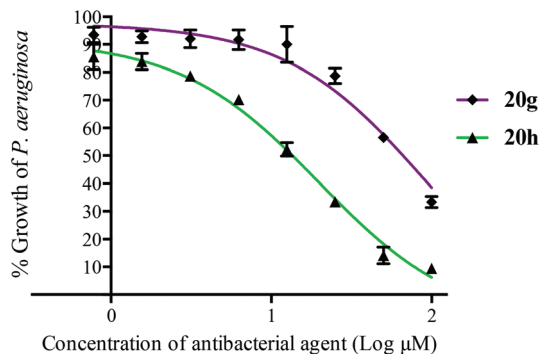


Fig. 2 The effects of argyrin A 20h and analogue 20g on the growth of amoxicillin-resistant *P. aeruginosa* PAO1 in Muller–Hinton broth at 13 h.

peptide and subsequent head-to-tail macrocyclization (Scheme 3). In this context, the (*S*)-Fmoc- β -phenylseleno cysteine **15** was used as a masked precursor for the dehydroalanine residue, and the thiazole dipeptide²⁸ was *N*-Boc protected (**16**) (see ESI†). Thus, the synthesized linear peptides **18** were subjected to PyBOP-mediated macrocyclization to yield **19**, followed by an oxidation–elimination reaction to afford the desired argyrin A **20h** and related analogues **20a–g**. The purity of the cyclic peptides were established by ¹H NMR and reversed-phase HPLC.

The antibacterial activity of a selection of argyrin analogues was evaluated against two Gram-negative bacteria, *Pseudomonas aeruginosa* PAO1 and *Proteus mirabilis* Hauser 1885 (ESI Table S1†). While the activity of argyrin A **20h** against *P. aeruginosa* PAO1 ($\text{MIC}_{50} = 19.8 \pm 1.6 \mu\text{M}$) is comparable to a reported value⁸ (Fig. 2), we were pleasantly surprised that the argyrin analogue **20g**, comprised of a (*S*)-5-methoxy-tryptophan residue, showed MIC_{50} of 90–100 μM against both bacteria. In contrast, the (*S*)-5-chloro- and (*S*)-5-methyl-tryptophan containing analogues **20e** and **20f** were essentially inactive. The experimental complex structure between bacterial EF-G and argyrin B shows a H-bond from Ser417 to the O of the 4-methoxy group in the Trp residue.⁷ Preliminary inspection of the complex structure suggests that either the H-bond is maintained but weaker due to an increased distance, or the 5-OMe is capable of H-bond interaction with Tyr683. However, argyrin analogues lacking a H-bond acceptor in a suitable position (**20e** and **20f**) were inactive.

Conclusions

A facile approach for the synthesis of enantiomerically pure (*S*)-tryptophan with halo-, methoxy- and alkyl-substitution has been established using inexpensive (*S*)-methylbenzylamine-based chiral auxiliary reagents. Using our range of new (*S*)-Trp analogues, we have also discovered that the antimicrobial activity of argyrin A, although dependent on the (*S*)-4-methoxy-tryptophan residue, tolerates a (*S*)-5-methoxy-tryptophan residue. We anticipate this will provide a useful platform for

the design and synthesis of more potent and species-specific antibacterial agent.

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Notes and references

- 1 E. Selva, L. Gastaldo, G. S. Saddler, G. Toppo, P. Ferrari, G. Carniti and B. P. Goldstein, *J. Antibiot.*, 1996, **49**, 145–149.
- 2 F. Sasse, H. Steinmetz, T. Schupp, F. Petersen, K. Memmert, H. Hofmann, C. Heusser, V. Brinkmann, P. von Matt, G. Hofle and H. Reichenbach, *J. Antibiot.*, 2002, **55**, 543–551.
- 3 P. Ferrari, K. Vekey, M. Galimberti, G. G. Gallo, E. Selva and L. F. Zerilli, *J. Antibiot.*, 1996, **49**, 150–154.
- 4 L. Vollbrecht, H. Steinmetz and G. Hofle, *J. Antibiot.*, 2002, **55**, 715–721.
- 5 I. Nিকেleit, S. Zender, F. Sasse, R. Geffers, G. Brandes, I. Soerensen, H. Steinmetz, S. Kubicka, T. Carlomagno, D. Menche, I. Guetgemann, J. Buer, A. Gossler, M. P. Manns, M. Kalesse, R. Frank and N. P. Malek, *Cancer Cell*, 2008, **14**, 23–35.
- 6 L. Buelow, I. Nিকেleit, A.-K. Girbig, T. Brodmann, A. Rentsch, U. Eggert, F. Sasse, H. Steinmetz, R. Frank, T. Carlomagno, N. P. Malek and M. Kalesse, *ChemMedChem*, 2010, **5**, 832–836.
- 7 B. Nyfeler, D. Hoepfner, D. Palestrant, C. A. Kirby, L. Whitehead, R. Yu, G. Deng, R. E. Caughlan, A. L. Woods, A. K. Jones, S. W. Barnes, J. R. Walker, S. Gaulis, E. Hauy, S. M. Brachmann, P. Krastel, C. Studer, R. Riedl, D. Estoppey, T. Aust, N. R. Movva, Z. Wang, M. Salcius, G. A. Michaud, G. McAllister, L. O. Murphy, J. A. Tallarico, C. J. Wilson and C. R. Dean, *PLoS One*, 2012, **7**, e42657.
- 8 P. Bielecki, P. Lukat, K. Huesecken, A. Doetsch, A. H. Steinmetz, R. W. Hartmann, R. Mueller and S. Haeussler, *ChemBioChem*, 2012, **13**, 2339–2345.
- 9 E. A. Villar, D. Beglov, S. Chennamadhavuni, J. A. Porco Jr., D. Kozakov, S. Vajda and A. Whitty, *Nat. Chem. Biol.*, 2014, **10**, 723–731.
- 10 (a) S. V. Ley, A. Priour and C. Heusser, *Org. Lett.*, 2002, **4**, 711–714; (b) G. Blaser, J. M. Sanderson, A. S. Batsanov and J. A. K. Howard, *Tetrahedron Lett.*, 2008, **49**, 2795–2798; (c) R. J. M. Goss and P. L. A. Newill, *Chem. Commun.*, 2006, 4924–4925.
- 11 J. Ma, W. Yin, H. Zhou, X. Liao and J. M. Cook, *J. Org. Chem.*, 2009, **74**, 264–273.
- 12 K. Harada, *Nature*, 1963, **200**, 1201.
- 13 W. Wu, Z. Li, G. Zhou and S. Jiang, *Tetrahedron Lett.*, 2011, **52**, 2488–2491.



- 14 R. H. Dave and B. D. Hosangadi, *Tetrahedron*, 1999, **55**, 11295–11308.
- 15 A. Arasappan, S. Venkatraman, A. I. Padilla, W. L. Wu, T. Meng, Y. Jin, J. Wong, A. Prongay, V. Girijavallabhan and F. G. Njoroge, *Tetrahedron Lett.*, 2007, **48**, 6343–6347.
- 16 A. R. Katritzky, B. Pilarski and L. Urogi, *Synthesis*, 1989, 949–950.
- 17 E. Vedejs and C. Kongkittigam, *J. Org. Chem.*, 2001, **66**, 7355–7364.
- 18 R. Baltzly and P. B. Russell, *J. Am. Chem. Soc.*, 1953, **75**, 5598–5602.
- 19 R. C. Bernotas and R. V. Cube, *Synth. Commun.*, 1990, **20**, 1209–1212.
- 20 M. Truong, F. Lecornue and A. Fadel, *Tetrahedron: Asymmetry*, 2003, **14**, 1063–1072.
- 21 D. Hazeldard, A. Fadel and C. Girard, *Tetrahedron: Asymmetry*, 2006, **17**, 1457–1464.
- 22 G. Brieger and T. J. Nestruck, *Chem. Rev.*, 1974, **74**, 567–580.
- 23 S. Ram and L. D. Spicer, *Tetrahedron Lett.*, 1987, **28**, 515–516.
- 24 J. Li, S. Wang, G. A. Crispino, K. Tenhuisen, A. Singh and J. A. Grosso, *Tetrahedron Lett.*, 2003, **44**, 4041–4043.
- 25 P. J. Kocienski, *Protecting groups*, Georg Thieme Verlag, New York, 2005.
- 26 S. D. Bull, S. G. Davies, G. Fenton, A. W. Mulvaney, R. S. Prasad and A. D. Smith, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3765–3774.
- 27 S. Kokinaki, L. Leondiadis and N. Ferderigos, *Org. Lett.*, 2005, **7**, 1723–1724.
- 28 S. V. Ley and A. Priour, *Eur. J. Org. Chem.*, 2002, 3995–4004.

