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Total synthesis and biological evaluation of simplified aplyronine analogues as synthetically tractable anticancer agents†

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The aplyronines are a family of highly cytotoxic marine natural products with potential application in targeted cancer chemotherapy. To address the severe supply issue, function-oriented molecular editing of their macrolactone scaffold led to the design of a series of simplified aplyronine analogues. Enabled by a highly convergent aldol-based route, the total synthesis of four analogues was achieved, with a significant improvement in step economy *versus* previous compounds, and their cancer cell growth inhibition in the HeLa cell line was determined. The modular strategy presented offers a means for significantly shortening their chemical synthesis to facilitate the continued development of this promising class of anticancer agent.

Isolated in 1993 by Yamada and co-workers from the sea hare *Aplysia kurodai*,¹ the aplyronines are a family of complex polyketides comprised of a 24-membered macrolactone and an elaborate side chain terminating in an *N*-vinyl-*N*-formamide moiety.^{1–3} Aplyronines A (1, Fig. 1) and C (2) bear a C29 *N,N*-dimethylalanine (DMAIa) residue, while aplyronine D (3) contains a *N,N*-dimethylglycine (DMGIy). Aplyronines A and D further feature a *N,N,O*-trimethylserine (TMSer) residue at C7. Aplyronine A was found to display sub-nanomolar growth inhibitory activity (mean GI₅₀ = 0.2 nM) in the NCI 60 cell line panel, prompting investigations into its mode of action in disrupting cytoskeletal dynamics.⁴ The 11-carbon side chain of the aplyronines is conserved across several related anticancer macrolides (reidispogiolide A (4), scytophycin C (5) and rhizopodin (6)), which all bind to actin as their protein target.⁵ Additionally, aplyronine A was found to bind to tubulin through the formation of an unprecedented actin–aplyronine–tubulin heterotrimeric complex.⁶ Interestingly, actin binding was shown

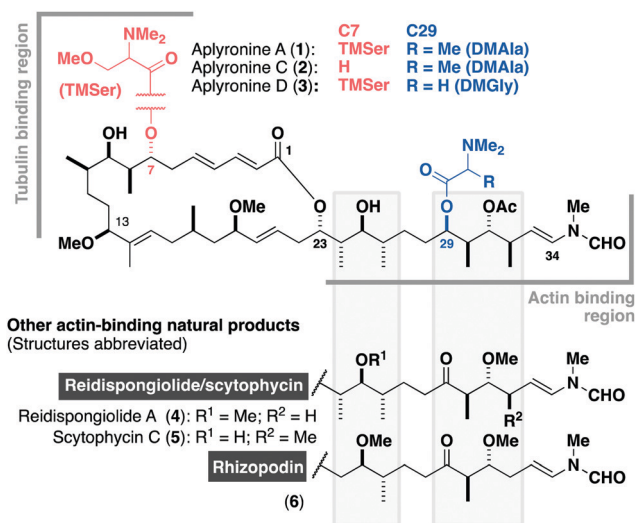


Fig. 1 The aplyronine family of cytotoxic marine macrolides, highlighting the structural differences between the congeners from C7 and C29 acyl substitution. Key regions implicated in protein binding are denoted, as well as their structural homology to related actin-binding macrolides.

to be a necessary but insufficient marker for their cytotoxicity, where aplyronine A is roughly 50 times more cytotoxic than aplyronine C, despite registering similar actin depolymerising ability. This structure–activity relationship (SAR) highlighted the synergistic role of the macrolactone, specifically the C1–C13 region, with the C7 TMSer important in forming the crucial interaction with tubulin in aplyronines A and D.⁷

Their extraordinary potency and novel mode of action mark the aplyronines out as promising candidates as novel payloads for antibody–drug conjugates.^{8,9} While recent total syntheses of the aplyronines have been achieved in 29–38 steps LLS (and up to 80 total steps),^{9,10} their structural complexity and natural scarcity (2.6 mg of aplyronine D was isolated from 300 kg of *Aplysia kurodai*)^{1b} adversely impact their further development. To address these challenges, we turned towards a function-oriented molecular editing of the aplyronine scaffold to help

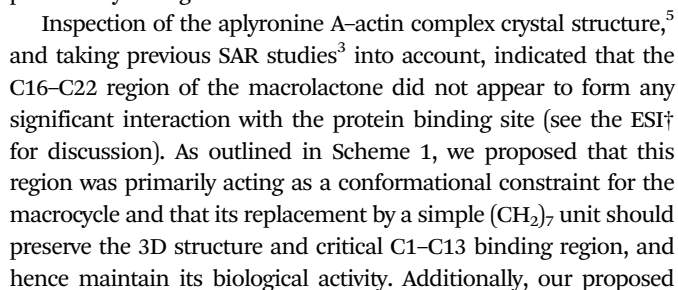
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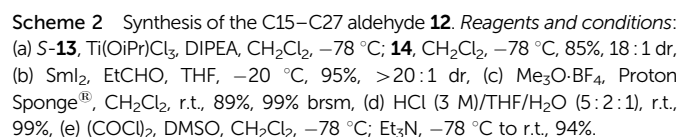
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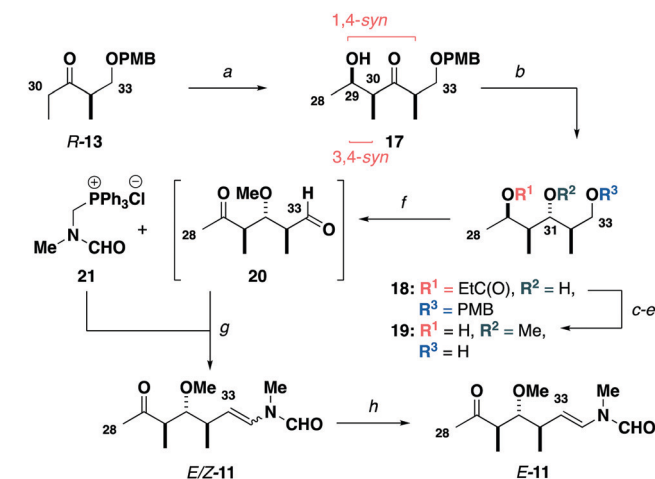
† Electronic supplementary information (ESI) available: Full experimental procedures and discussion. See DOI: 10.1039/c9cc09050a

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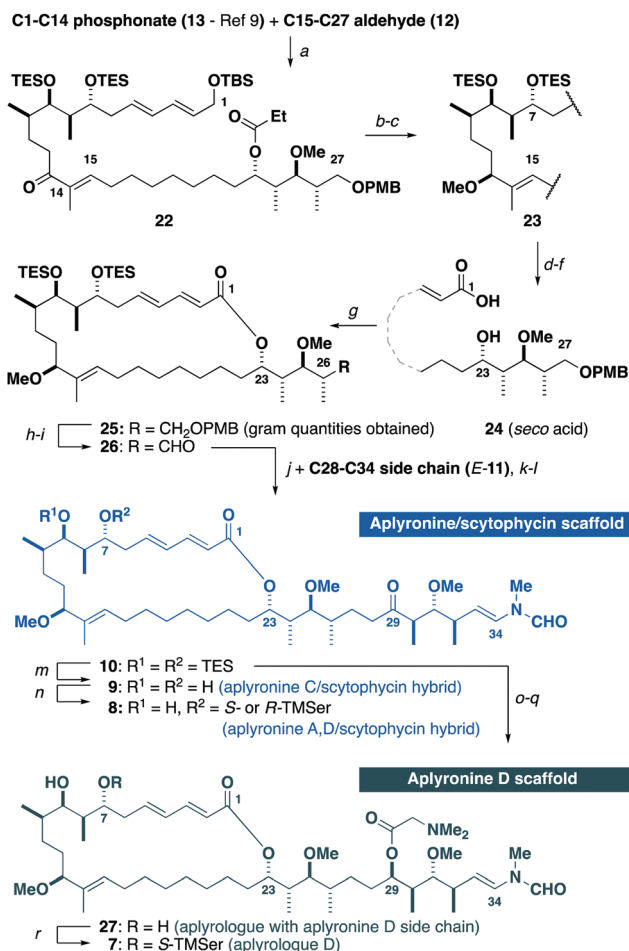
Synthesis of the modified C28–C34 side chain (Scheme 3) commenced with an analogous titanium aldol reaction between the ketone *R*-**13** and acetaldehyde to give **17**. Adduct **17** was reduced to give alcohol **18** followed by methylation, PMB ether cleavage and ester reduction to afford the diol **19**. At this point, a double oxidation under Swern conditions gave ketoaldehyde **20**, which was directly subjected to a Wittig olefination¹⁶ with phosphonium salt **21** to generate the enamides *E/Z*-**11**. Final completion of the configurationally pure C28–C34 ketone *E*-**11** was accomplished by I₂-mediated isomerisation.





With all three fragments in hand, attention now turned towards the assembly of the full carbon skeleton of the targeted analogues. Aldehyde **12** was first coupled with the phosphonate **13** (10 steps)^{9a} via an HWE olefination¹⁷ to generate the C1–C27 fragment **22** (Scheme 4). A CBS reduction¹⁸ of the C13 ketone was then followed by methylation to give **23**. Next, the C23 ester was cleaved and a DDQ-mediated deprotection/allylic oxidation¹⁹ afforded the *seco* acid **24**. The macrolactonisation reaction of **24** proceeded smoothly under Yamaguchi conditions²⁰ to give **25**. Moving forward, oxidative cleavage of the PMB ether in **25** followed by Dess–Martin oxidation of the resulting alcohol afforded the aldehyde **26**. This was then subjected to a three-step sequence involving a Cy₂BCl-mediated aldol coupling^{9b} with the C28–C34 ketone **E-11**, a Burgess elimination followed by a copper-catalysed conjugate reduction of the resulting enone gave **10**, corresponding to a protected aplyrologue bearing the scytophycin/reidispongolide-type side chain. Additionally, the C29 ketone in **10** could be reduced and acylated with DMGly under Keck conditions²¹ to give the 1,3-diol **27** after deprotection, which corresponded to the aplyrologue scaffold bearing the aplyronine-type side chain. The advanced intermediate **10** was also deprotected to give the scytophycin–aplyronine C hybrid **9**. In turn, this could be site-selectively acylated at C7 with the required enantiomer of TMSer to generate the scytophycin–aplyronine A hybrids **S-8** and **R-8**. Noting that aplyronine D bearing the C7 TMSer and C29 DMGly residues was reported to be the most active congener,^{1b} the corresponding aplyrologue D was targeted. Thus, the diol **27** was site-selectively acylated with *S*-TMSer to generate **7**.

With this first series of aplyrologues in hand, attention now turned towards determining their *in vitro* cytotoxicity. It was considered prudent to initially examine the activity of our previously synthesised samples of aplyronines A (**1**), C (**2**) and D (**3**), with discodermolide included as a control (Table 1).



While the expected sub-nanomolar potencies were found for aplyronines A and D, we did not find any significant difference between these congeners in the same HeLa cell line (Table 1, entries 1–3). However, our synthetic aplyrologues did not register the same exquisite potency compared with the native aplyronines. Nevertheless, the compounds *R*- and *S*-**8** (entries 5 and 6), bearing the scytophycin side chain, as well as **7** (entry 4) still gave inhibitory activities in the nM range. Interestingly, both the configuration of the TMSer residue and the nature of the C29 functionality as the ketone or DMGly ester did not appear to greatly influence the biological activity, suggesting that the deleterious effect may be attributable to an oversimplification of the southern hemisphere region of the macrolactone.

Table 1 Antiproliferative activity of synthetic aplyronines and aplyrologues against the HeLa cell line (discodermolide was included as a control)

Entry	Compound	IC ₅₀ ^a (nM)
1	Aplyronine A (1)	0.31
2	Aplyronine C (2)	3.67
3	Aplyronine D (3)	0.26
4	7 (aplyrologue D)	124
5	S-8 (aplyronine A, D/scytophycin C hybrid)	164
6	R-8 (aplyronine A, D/scytophycin C hybrid)	192
7	9 (aplyronine C/scytophycin C hybrid)	>1000
8	Discodermolide	3.40

^a *n* = 3. See the ESI for cytotoxicity curves and further experimental details.

In conclusion, the preliminary biological results obtained for this first series of aplyrologues suggests that the southern hemisphere of the macrocycle may play a subtle but vital role in reinforcing the proposed actin–tubulin interaction that the aplyronines uniquely mediate. Additionally, the biological data obtained for our synthetic aplyronines does not replicate the greater cytotoxicity previously reported for a natural sample of aplyronine D relative to aplyronine A,^{1b} signifying the scope for structural diversification of the side chain. In future work, further judicious molecular editing of the southern hemisphere region can be flexibly adapted towards the incorporation of more elaborate macrocyclic tethers. We envisage that our modular synthetic platform offers a means for enhancing the synthetic tractability of the aplyronines to facilitate the continued development of this promising class of anticancer agent.²²

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Conflicts of interest

There are no conflicts to declare.

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