Efficient, Divergent Synthesis of Cryptophycin Unit A Analogues

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Abstract

A flexible and divergent synthesis of cryptophycin unit A analogues is described. This method relies on iridium-catalysed stereo- and enantioselective crotylation and chemoselective one-pot oxidative olefination to access common intermediate **8**. Heck, cross metathesis, and Suzuki-Miyaura reactions are illustrated for the generation of methyl ester unit A analogues **10a-d**.

The cryptophycins are a family of lichen-derived cyanobacterial peptolides¹⁻³ that represent some of the most potent microtubule stabilising agents discovered to date. Effective at levels significantly lower than either vinblastine or paclitaxel, cryptophycins have proven to be powerful cytostatic agents capable of arresting the growth of P-glycoprotein-expressing multidrug resistant (MDR) human carcinoma cell lines.^{4,5} However, pronounced neurotoxicity precluded development of cryptophycin **52** (Figure 1), a synthetic analogue of cryptophycin **1** that had been identified as the most promising clinical candidate.⁶ As a result, extensive medicinal chemistry efforts have been undertaken in the search for new analogues with improved therapeutic profiles.^{7,8}

Of the four subunits that comprise the cryptophycins (Figure 1), unit A is the only subunit where significant structural modification has been performed and shown to be tolerated.⁹⁻¹¹ As noted by Corbett and coworkers,¹² substituted phenyl ring analogues of unit A have displayed antitumor activity with promising pharmacological profiles. Therefore, we were motivated to establish a selective, scalable, and efficient method for the production of unit A analogues varied in the phenyl region to support development of improved cryptophycins for the treatment of cancer.

We initially envisioned a synthetic route modelled closely on that of Eggen et al.¹³ (Scheme 1), which was attractive for utilising Heck coupling for the late-stage installation of the phenyl ring in unit A. This coupling was ideal for derivatization of the unit A phenyl ring using diverse aryl iodides. However, development of this route in our laboratory for higher throughput proved to be problematic. The Brown crotylboration was the most significant hindrance to large-scale synthesis, as it proceeded in poor yield with competing decomposition of aldehyde **I-3**. In addition, protecting group exchange (**I-4** to **I-6** and **I-8** to **I-9**) was inefficient, and the second round of oxidation provided a volatile silyl ether aldehyde (**I-6**) whose isolation was problematic. The complexity and low-throughput (0.12% over 11 steps to **I-9**; data not shown) of this route hindered our ability to access the desired quantities of unit A and its analogues.

Facing these initial obstacles, we sought a new route to unit A analogues that was both short and enabled diversification as a terminal, divergent step. Recent advances in crotylation technology pioneered in the Krische laboratory¹⁴⁻¹⁷ created the opportunity for a catalytic process with the potential for recovery and recycling of the expensive iridium-based reactive complex.¹⁸ This crotylation was also highly efficient, as it eliminated the need for a separate aldehyde generation and isolation step. Furthermore, an Eli Lilly process chemistry report¹⁹ suggested that tedious protection and deprotection steps through the final alcohol oxidation would not be necessary, and that an *in situ* olefination could further shorten the synthetic route. Thus, we sought to pursue analogue synthesis through olefin cross metathesis (CM), Heck coupling, and Suzuki-Miyaura cross coupling.

Our synthesis of intermediate **8**, which would serve as the common intermediate for unit A and analogues, is summarised in Scheme 2. Starting from 1,3-propanediol, monosilylated alcohol **4** was prepared as previously reported in excellent yield.²⁰ Treatment of **4** with the Ir complex **5**, prepared and isolated as described,¹⁶ in the presence of *n*-methyl allyl acetate afforded the expected crotyl alcohol **6** in a 6:1 dr that was sufficiently pure to carry forward without purification. Mosher ester analysis^{21,22} of the diastereomeric mixture of **6** confirmed the correct stereochemistry (3*S*,4*R*) and revealed a 95:5 er.[‡] Precipitation with pentane and filtration allowed recovery of the catalyst, while the filtrate containing **6** was concentrated and treated with TBAF to liberate crotyl diol **7** in good yield from **4**. With **7** in hand, a one-pot oxidation/olefination sequence was employed to effect chemoselective conversion of the primary alcohol to unsaturated ester **8** (>20:1 *E/Z*).²³ The terminal alkene on **8** was then employed as the handle to generate unit A analogues.

A series of phenyl moieties (4-H, 4-trifluoromethyl, 4-methoxy, and 4-cyano) was chosen to demonstrate the flexibility of the reported synthetic scheme. This series was developed for the wide range of electron demand presented and for structural replication of non-native crotophycins produced through precursor-directed biosynthesis.³

As previous methods utilised Heck coupling for appending the phenyl on unit A, this was the first route explored for analogue creation (Table 1). Phosphine-free Pd catalysis with aryl iodides **9a-d** in acetonitrile proved to be the most versatile for reactions with the terminal olefin of **8**, but also likely to induce reactivity with the α,β -unsaturated methyl ester.²⁴⁻²⁶ Decomposition was evident, likely due to extended heating times, but yields were acceptable for analogues **10a-d**.

The second route investigated for flexible analogue generation was CM (Table 2). In this case, olefin **8** underwent metathesis in the presence of Hoveyda-Grubbs II catalyst in dichloromethane at reflux temperature with a set of *para*-substituted styrenes (**11a-d**).²⁷⁻²⁹ CM was successful in providing the desired analogues **10a-d**, but competitive insertion of the α,β -unsaturated methyl ester prevented higher yields (Table 2). However, we expect this route could be further optimised to utilise the full spectrum of styryl building blocks available.

The final route developed for unit A analogue synthesis employed Suzuki-Miyaura cross coupling. For this method, the boronic ester **12** was first prepared through CM (Table 3).^{30,31} Aryl iodides **9a-d** were then coupled with **12** using Pd₂(dba)₃ in a mixture of water and glyme to afford analogues **10a-d** in good yield regardless of the presence of electron donating or withdrawing substituents (Table 3).³² Reactivity could also be achieved with

[‡]The MTPA ester was synthesised from purified **6** using (*S*)-(+)-MTPA acid chloride in anhydrous pyridine at 60 °C for 24 h. The ¹⁹F NMR spectra of esters prepared from **6** and its enantiomer (from (*S*)-(-)-**5**) were compared to determine the er (Supporting Information).

aryl bromides and chlorides with increased heating times and temperatures, proving this Suzuki-Miyaura route to be the most efficient for future analogue generation.

With effective elaboration of **8** via Heck reaction, CM, and Suzuki-Miyaura coupling, the remaining transformation involved hydrolysis of diverse methyl esters **10a-d** to the acids **13a-d**. This was accomplished with LiOH in a 1:1:0.5 mixture of methanol/water/tetrahydrofuran (Scheme 3). The presence of the free acid now allows attachment of these novel unit A analogues to linear depsipeptide precursors of cryptophycin.³³

In summary, we have established a short and efficient synthesis to intermediate **8** (4 steps, 34% overall yield) from which flexible coupling methods, particularly Suzuki-Miyaura cross coupling, provide access to a broad range of phenyl or more highly functionalised aromatic moieties in the synthesis of unit A analogues. With this strategy in place, the remaining objective involves coupling of the analogues to units B, C, and D to generate seco-cryptophycin intermediates. Our laboratory has previously demonstrated that solid phase synthesis³⁴ followed by Crp thioesterase and epoxidase-mediated chemoenzymatic assembly^{3,35,36} enable rapid production of cryptophycin anticancer agents. We expect the versatile unit A synthetic strategy reported herein will open new opportunities to produce unique cryptophycin analogues for on-going drug development efforts.

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Supplementary Material

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Figure 1.
Cryptophycins 1 and 52 with subunits A, B, C, and D highlighted.

Scheme 1.

Original synthetic route to unit A and analogues developed from Eggen et al.

Scheme 2.

Our efficient synthesis developed for common intermediate **8**.

Scheme 3.

Hydrolysis of methyl esters **10a-d** to acids **13a-d**.

Table 1Heck route to unit A analogues.^a

a: R = H	b: R = CF ₃	c: R = OMe
d: R = CN		

Aryl iodide	Product	Yield (%) ^b
9a	10a	31
9b	10b	28
9c	10c	38
9d	10d	30

^a Reaction conditions: Pd(OAc)₂ (1.6 mol%), 1.1 eq. aryl iodide, 10 eq. Et₃N, CH₃CN, 85 °C, 18 h.^b Isolated yield.

Table 2CM route to unit A analogues.^a

Styryl fragment	Product	Yield (%) ^b
11a	10a	30
11b	10b	23
11c	10c	26
11d	10d	8

^aReaction conditions: Hoveyda-Grubbs II (5 mol%), 4.0 eq. styryl fragment, DCM, reflux, 2 h.^bIsolated yields.

Table 3

Suzuki-Miyaura cross coupling to unit A analogues.^b

Aryl iodide	Product	Yield (%) ^b
9a	10a	42
9b	10b	55
9c	10c	53
9d	10d	63

^aReaction conditions: [Pd₂(dba)₃] (2 mol%), PCy₃ (4.8 mol%), 1.7 eq. K₃PO₄, DME/H₂O (2:1), 85 °C, 2 h.

^bIsolated yields from **12**.