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Total Synthesis of the Cyclic Depsipeptide YM-280193, a Platelet Aggregation Inhibitor

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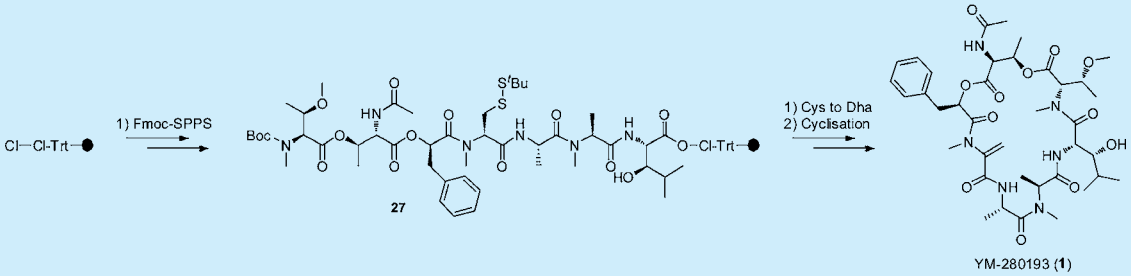
Supporting Information



ABSTRACT: The first total synthesis of YM-280193, a cyclic depsipeptide that inhibits the ADP-induced aggregation of human platelets, is described. The monomer and dipeptide fragments were prepared using conventional chemistry and subsequently assembled by Fmoc-solid-phase peptide synthesis (Fmoc-SPPS). A late-stage novel bis-alkylation−elimination of cysteine on-resin was employed to introduce the unnatural *N*-methyldehydroalanine moiety. The final step involved execution of a key macrolactamization reaction between the hindered unnatural *N*,*O*-dimethylthreonine and *β*-hydroxyleucine residues.

Cardiovascular diseases are a major cause of morbidity and the leading cause of mortality worldwide, accounting for 30% of total deaths, approximately 17 million annually.[1] Of these thrombotic complications such as heart attacks and stroke resulted in 7.6 and 5.7 million deaths, respectively.[1] Currently, platelet aggregation inhibitors such as orally available aspirin and clopidogrel constitute the largest class of antithrombotic drugs that are used to treat and manage thrombotic complications.[2] The effectiveness of available antithrombotics, however, is limited by a high degree of interindividual variation in efficacy and safety, breakthrough thrombosis, and recurrent ischemic events.[2,3] These limitations and the prevalence of cardiovascular diseases have contributed to the continuous need to develop new antithrombotic drugs.[2]

Recently, Taniguchi et al.[4] discovered four novel cyclodepsipeptides, namely YM-280193 (**1**), YM-254890 (**2**), YM-254891 (**3**), and YM-254892 (**4**) with platelet aggregation inhibitory activity from the Japanese soil microorganism *Chromobacterium* sp. QS3666 (Figure 1).[4,5] The four cyclodepsipeptides **1**−**4** share the common cyclic core of **1**, which consists of an unnatural *β*-hydroxyleucine (*β*-Hyleu) residue, D-phenyllactic acid (D-Pla), three *N*-methylated residues, namely *N*,*O*-dimethylthreonine (*N*,*O*-Me$_2$Thr), *N*-methylalanine (*N*-MeAla), and *N*-methyldehydroalanine (*N*-MeDha), an *N*-acetyl-L-threonine residue, and naturally occurring L-alanine.

In addition to the core backbone of **1**, octadepsipeptides **2**−**4** have an extra *β*-hydroxyleucine residue attached that is *N*-acylated with an *N*-acetyl, *N*-propionyl, or *N*-methylthioacetyl group, respectively. Evaluation of ADP-induced platelet aggregation inhibitory activity in human platelet-rich plasma revealed that octadepsipeptides **2**−**4** exhibited potent IC$_{50}$ values of 0.26, 0.27, and 0.29 *μ*M, respectively.[5] In contrast, heptadepsipeptide **1** displayed reduced potency with an IC$_{50}$ of 3.4 *μ*M, which suggests that the additional *β*-hydroxyleucine residue is critical for activity; however, steric allowance at the *N*-acyl group is also tolerated.[5]

More importantly, further research proved YM-254890 (**2**) to be the first and currently only known selective inhibitor of

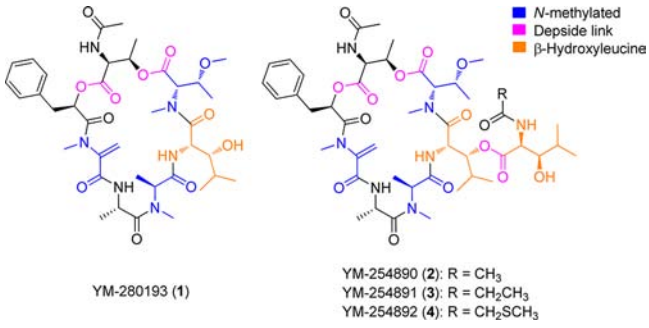


Figure 1. Structures of YM-280193 (**1**), YM-254890 (**2**), YM-254891 (**3**), and YM-254892 (**4**).

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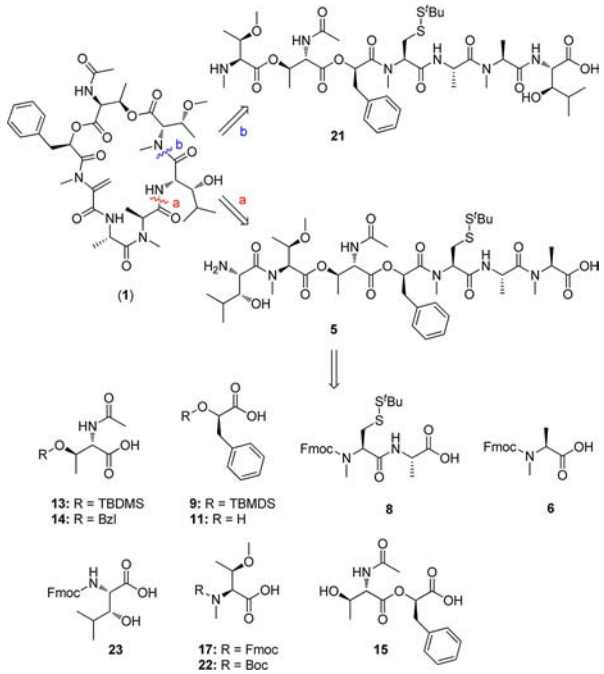
$G\alpha_{q/11}$, a G protein predominantly expressed in human platelets that is crucial to platelet activation and aggregation.[6−8] Hence, in addition to the search for new antithrombotics, YM-254890 (**2**) is also an invaluable probe for understanding G_q-mediated biological processes. Unfortunately, despite the biological importance of YM compounds **1**−**4**, none of these YM molecules are commercially available to researchers. Demand for the YM depsipeptides has been highlighted, for example, by a $100000 USD reward for the total synthesis of 1 mg of YM-254890 (**2**), in which 228 applicants worldwide actively participated.[9] However, the total synthesis of **2** remains elusive, as the reward was later withdrawn without announcement of a winning solution. Intrigued by the platelet aggregation inhibitory activity of these YM compounds **1**−**4**, their synthetically challenging cyclodepsipeptide structure, and the lack of availability of any of these compounds **1**−**4** to researchers, we herein report the first total synthesis of YM-280193 (**1**), the *cyclo*-heptadepsipeptide core of the more potent YM-molecules **2**−**4**.

We chose to construct **1** using a hybrid of solution-phase synthesis to create the protected building blocks followed by Fmoc-solid-phase peptide synthesis (Fmoc-SPPS) to assemble the linear peptide precursor (Scheme 1). The use of Fmoc-SPPS

Scheme 1. Initial (Route a) and Final (Route b) Retrosynthetic Disconnections of YM-280193 (1) and Building Blocks

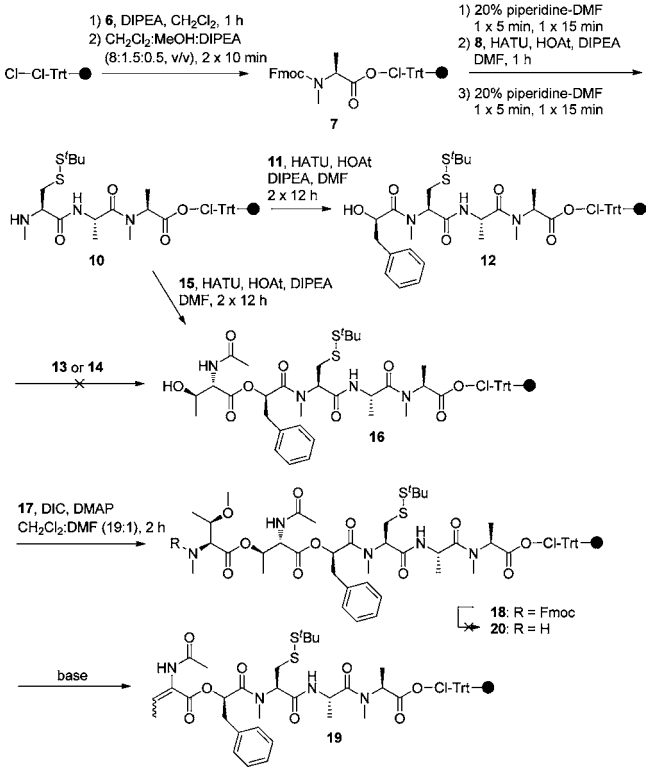


for the preparation of the linear precursor eliminates the necessity for purification and isolation of intermediates during the synthesis and also overcomes poor solubility issues associated with protected peptide fragments during solution-phase condensation.[10] However, Fmoc-SPPS would render the dehydroalanine (Dha) residue of **1** susceptible to Michael addition from nucleophiles such as piperidine which results in the formation of β-piperidyl adducts.[11,12] Typically, Dha is masked during SPPS and introduced at a late stage via either the activation and elimination of serine derivatives[13,14] or oxidative elimination of alkylated cysteines[15,16] and selenocysteines.[17,18] In our case, we chose to mask the Dha residue of **1** as a suitably protected cysteine due to the prevalence of other competing β-hydroxy amino acids in **1**.

Choice of an appropriate site to effect the key macrocyclization step was crucial. Disconnection of **1** at the two macrolactonization sites[19] was excluded as well as the three sites that result in an *N*-methylated terminal linear precursor.[20] The *N*-MeDha-Ala junction which results in a masked *C*-terminal cysteine residue that is prone to *C*-terminal racemization and conversion to Dha during Fmoc-SPPS was also not appropriate.[12,21] The initial disconnection site was thus chosen at the *N*-MeAla-Hyleu junction, leading to linear precursor **5** composed of an *N*-terminal β-Hyleu and a *C*-terminal *N*-MeAla residue (Scheme 1, route a). Moreover, the high number of *N*-methylamino acids were anticipated to contribute to effective cyclization by minimizing the head to tail spatial proximity through introducing *cis*-amide bonds and inducing β-turns.[20]

Fmoc-SPPS to access acyclic peptide **5** commenced with attachment of the *C*-terminal Fmoc-*N*-MeAla-COOH (**6**) residue onto 2-chlorotrityl chloride (2-CTC) resin (Scheme 2). N^{α}-Fmoc-deprotection of resin-bound **7** and sequential

Scheme 2. Initial Synthetic Strategy toward Linear Precursor 5



elongation with successive residues met with significant diketopiperazine (DKP) formation despite the use of the sterically hindered 2-chlorotrityl linker and treatment with the milder base piperazine (5% in DMF) containing 0.1 M 6-Cl-HOBt as an acidic additive.[21−23] Consequently, the second and third residues were introduced as the preformed dipeptide Fmoc-*N*- MeCys(S^tBu)-Ala-COOH (**8**) (see the Supporting Information).

Briefly, dipeptide **8** was synthesized by direct *N*-methylation of Boc-Cys(S^tBu)-COOH followed by HCTU-mediated condensation with NH_2-Ala-CO_2Me. Ester hydrolysis, Boc-deprotec-

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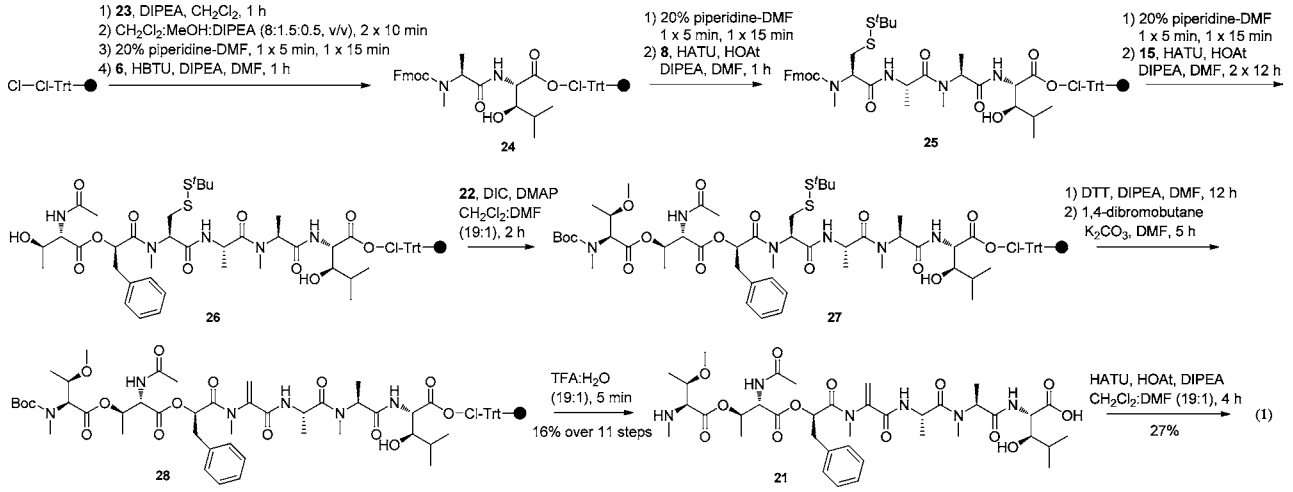
Scheme 3. Synthesis of YM-280193 (1)



tion, and Fmoc-reprotection furnished the desired dipeptide fragment **8**. Pleasingly, introduction of Fmoc-dipeptide **8** in lieu of sequential elongation successfully circumvented DKP formation (Scheme 2).

Next, N^{α}-Fmoc-deprotection and coupling of TBDMSO-D-Pla-COOH (**9**) onto resin-bound *N*-MeCys **10** using a variety of peptide coupling reagents and conditions for *N*-alkylated residues was slow resulting in incomplete peptide bond formation. Nevertheless, the poor reactivity of resin-bound *N*-MeCys **10** proved advantageous as HO-D-Pla-COOH (**11**) could be coupled without protection of its hydroxyl functionality using HATU and HOAt to give **12**, thereby avoiding an extra protection and deprotection step (Scheme 2). Subsequent esterification of resin-bound tetrapeptide **12** with the required *N*-acetylated derivatives Ac-Thr(TBDMS)-COOH (**13**) or Ac-Thr(Bzl)-COOH (**14**) was then extensively investigated using either Steglich or modified Yamaguchi conditions,[24] acyl halides, traditional mixed anhydride reagents, or the addition of Li salts[25] with extended reaction time at room temperature or with microwave irradiation, but all proved fruitless (Scheme 2).

Because of the problematic on-resin esterification of **12**, the fourth and fifth residues were introduced as the preformed side-chain unprotected dipeptide Ac-Thr(OH)-D-Pla-COOH (**15**) (see the Supporting Information). Briefly, solution-phase esterification of Boc-Thr(Bzl)-COOH with HO-D-Pla-CO_2Bn using DCC/DMAP afforded the terminally protected depsipeptide in almost quantitative yield. Global deprotection via Pd/C catalyzed hydrogenolysis and acid-mediated N^{α}-Boc-removal followed by *N*-acetylation of the free amine furnished the desired ester fragment **15**. Gratifyingly, coupling of fragment **15** onto resin-bound *N*-MeCys **10** using HATU and HOAt afforded pentadepsipeptide **16** (Scheme 2). Consequent on-resin esterification of pentapeptide **16** with Fmoc-*N*,*O*-Me_2Thr-COOH (**17**) using DIC/DMAP afforded Fmoc-hexadepsipeptide **18** (Scheme 2). N^{α}-Fmoc-deprotection of depsipeptide **18**, however, led to complete α,β-elimination at the *N*-AcThr residue forming the dehydrobutyrine-pentapeptide byproduct **19** and no desired Fmoc-deprotected **20** (Scheme 2).[12,21]

Having established that depsipeptide **18** was stable to acid but unstable to base, the disconnection site was repositioned to the *N*,*O*-Me_2Thr-β-Hyleu junction, forming linear precursor **21** (Scheme 1, route b). This allows the use of Boc-*N*,*O*-Me_2Thr-COOH (**22**) (see the Supporting Information) instead of Fmoc-*N*,*O*-Me_2Thr-COOH (**17**), as the *N*-terminal Boc group is conveniently removed with TFA during resin cleavage. For our purposes, the *C*-terminal Fmoc-β-Hyleu(OH)-COOH (**23**) residue was synthesized using Belokon's nickel complex chemistry[26] and used herein with its secondary hydroxyl group unprotected (see the Supporting Information).

Thus, linear precursor **21** was synthesized using our previously optimized conditions which began by anchoring Fmoc-β-Hyleu(OH)-COOH (**23**) onto 2-CTC resin (Scheme 3). N^{α}-Fmoc-deprotection using piperidine-DMF followed by HBTU-mediated coupling of Fmoc-*N*-MeAla-COOH (**6**) afforded resin-bound dipeptide **24**. N^{α}-Fmoc-deprotection of **24** and subsequent coupling of dipeptide Fmoc-*N*-MeCys(S^{t}Bu)-Ala-COOH (**8**) was successfully effected using HATU and HOAt, which are excellent reagents for effecting coupling with *N*-methylated residues.[11] N^{α}-Fmoc-deprotection of **25** resulted in racemization at the *N*-MeCys residue even when milder deprotection conditions were used, but this is tolerated in our synthetic strategy as Cys is eventually converted to the achiral Dha residue. Subsequent double couplings of unprotected dipeptide Ac-Thr(OH)-D-Pla-COOH (**15**) using HATU and HOAt furnished depsipeptide **26**. Next, selective esterification of Boc-*N*,*O*-Me_2Thr-COOH (**22**) at the *N*-AcThr residue of resin-bound depsipeptide **26** using DIC/DMAP provided depsipeptide **27** with a masked cysteine residue as a substitute for Dha.

Inspired by the solution-phase conversion of cysteine to Dha via bis-alkylation−elimination reported by Davis et al.[27] and previous work on the elimination of labile sulfonium ions to dehydroalanine,[28,29] we applied this strategy to the solid-phase synthesis of dehydroalanyl peptide **28**. To the best of our knowledge, this is the first application of this methodology to the solid-phase synthesis of dehydroalanine-containing peptides. Reduction of the *S*-*tert*-butylthio group of heptapeptide **27** with dithiothreitol (DTT) and DIPEA afforded the free thiol peptide.[30] Pleasingly, bis-alkylation of the sulfhydryl functionality with 1,4-dibromobutane and elimination of the labile sulfonium salt with potassium carbonate afforded resin-bound dehydropeptide **28**.

Next, dehydropeptide **21** was released from 2-CTC resin by treatment of resin-bound **28** with trifluoroacetic acid, which concomitantly removed the *N*-terminal Boc group. RP-HPLC purification of unprotected depsipeptide **21** was then followed by a difficult macrocyclisation between the *N*-terminally methylated *N*,*O*-Me_2Thr residue and the *C*-terminal sterically hindered β-Hyleu residue. The key macrolactamisation was conducted

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Org. Lett. 2015, 17, 492−495

under high dilution solution phase conditions by slowly adding a mixture of linear precursor **21**, HATU, and HOAt in CH_2Cl_2/DMF (9:1) to a stirring solution of DIPEA in CH_2Cl_2. LC-MS analysis of a crude aliquot after addition of the coupling mixture indicated rapid consumption of the linear peptide and the formation of two closely eluting products in a 1:2 ratio, both of which exhibited the correct mass of the cyclic product. Prolonged heating at 50 °C of an aqueous acetonitrile solution of these products with monitoring by LC−MS indicated that the two products were stable to interconversion. This suggested that the cyclic products were likely to be epimers resulting from *C*-terminal epimerization during macrocyclisation rather than *cis*−*trans* rotational isomers at the *N*-methylated amide bonds. However, our attempts to improve this ratio by modifying either the base, coupling reagents or solvent were all unsuccessful.

Subsequent RP-HPLC purification of the reaction mixture suggested the earlier eluting product to be YM-280193 (**1**), as its ^{1}H NMR spectrum exhibited an analogous mixture of conformers (10:2, CD_3CN) present in naturally occurring **1**. Furthermore, both the ^{1}H and ^{13}C spectroscopic data of the major conformer of synthetic **1** were in agreement with the tabulated major conformer signals of isolated (**1**) (see the Supporting Information).[5] Synthetic **1** was characterized by LC−MS with purity >95%, and HRMS confirmed formation of the desired macrolactam product (calculated and observed mass of 811.3854 and 811.3857 respectively, see the Supporting Information). Moreover, IR spectral analysis of synthetic **1** was in agreement with the reported data of isolated **1**, and comparable optical rotation of synthetic **1** $[\alpha]_D$ −52.0 (*c* 0.08, MeOH) with isolated **1** $[\alpha]_D$ −61.3 (*c* 0.30, MeOH) further verified the elucidated structure of the natural product.[5] Initial biological evaluation of synthetic **1** suggested some activity; however, we have insufficient material to provide statistically significant results at this stage. Regrettably, the native material was not available for direct comparison.

In conclusion, we herein report the first total synthesis of YM-280193 **1**, using a combination of solution and solid-phase synthesis. The monomer and dipeptide fragments were prepared using conventional chemistry and subsequently assembled on 2-CTC resin. The low reactivity of the side-chain hydroxyl groups of Ac-Thr(OH)-D-Pla-COOH (**15**) and Fmoc-*β*-Hyleu(OH)-COOH (**23**) allowed us to incorporate these residues with minimal protection during Fmoc-SPPS. Pleasingly, the *N*-MeDha residue was installed selectively demonstrating the first on-resin application of bis-alkylation−elimination of cysteine in Fmoc-SPPS. The powerful coupling reagent HATU was used for both solid-phase couplings onto the poorly reactive *N*-methylated residues and for the challenging solution-phase macrolactamization reaction between the hindered *N*-terminal *N*,*O*-Me_2Thr and the *C*-terminal *β*-Hyleu residues. We envisage that further side-chain esterification of this core cyclic backbone (**1**) with an appropriately protected *β*-Hyleu residue will afford the more potent YM-molecules **2**−**4** in due course. Importantly, good agreement of the spectroscopic data (^{1}H NMR, ^{13}C NMR, HRMS, IR) and optical rotation of synthetic **1** with naturally occurring **1** confirms the structure of YM-280193 elucidated by Taniguchi et al.[5]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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