Function-Oriented Synthesis toward Peloruside A Analogues

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ABSTRACT: A convergent and rapid synthesis of original C2,C3-unsaturated, C11,C13-keto–enol macrocycles with a peloruside A skeleton has been developed. These original unsaturated macrocycles constitute valuable platforms to access peloruside A analogues with high diversity. The four-fragment strategy implemented features two aldol-type couplings with the central C12–C14 building block TES-diazoacetone and a late-stage ring-closing metathesis. Enantiopure analogue 18ab showed antiproliferative activity in the low micromolar range on NCI and MCF7 tumor cell lines.

Peloruside A (PelA) is a highly cytotoxic 16-membered macrolide, extracted in 2000 from the New Zealand marine sponge Mycale hentscheli (Figure 1). Natural congener peloruside B was later discovered, displaying similar cytotoxicity. Like paclitaxel, PelA acts as a potent microtubule stabilizing agent, stopping cell division in the G2/M phase. Due to its unique nontaxoid binding-site on tubulin β, PelA displays exciting properties like synergistic effects with taxanes and preserved efficiency in paclitaxel and epothilone-resistant cell lines. More recently, PelA proved highly efficient in targeting tumoral angiogenesis. Preliminary investigations on its potential role on neurodegenerative and autoimmune disorders also led to encouraging results. Despite its promising pharmacological profile, preclinical trials on PelA stagnate due to short supply of the natural product.

Six total syntheses have been achieved so far, providing enantiopure peloruside A on a milligram scale. Around 20 analogues were reported, including natural congeners, analogues produced by hemisynthesis or resulting from total synthesis. Our previous work in this field had resulted in the convergent total synthesis of a simplified C2,C3-unsaturated analogue, displaying antiproliferative activity around 15 μM on three tumor cell lines. The available data on the structure–activity relationship (SAR) of PelA have been recently reviewed. Key structural elements have emerged, including the primary alcohol of the lateral chain and the embedded tetrahydropyran skeleton, though the influence of each

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Figure 1. Structures of the marine sponge macrolides peloruside A (PelA) and peloruside B.

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first, C15−C20 fragment 1 and C12−C14 fragment triethyilslyldiozoacetone 2 (TES-diazoacetone) would be coupled by "methyl-side" aldolization (step 1), followed by esterification of cinnamic acid (C1−C2 fragment 3) with the resulting C15 alcohol (step 2). Assembly of C3−C11 fragment 4 would be carried out by "diazo-side" addition (step 3), followed by dediazotation/1,2-H migration (step 4). A final RCM step would lead to the expected C2−C3 unsaturated macrocycles (step 5). This "diazo route" illustrates the synthetic potential of the sequential double cross-aldolization methodology we had previously developed on TES-diazoacetone.14 Both the C11,C13 keto−enol moiety and the C2,C3 double bond constitute an attractive gateway to elaborating a stable C2,C3-unsaturated macrocyclic chemical instability. Functionalization of these fragments was not biologically evaluated due to chemical instability.

In the present work, we describe a four-fragment-based approach for the function-oriented synthesis of PelA analogues via the elaboration of stable C2,C3-unsaturated macrocyclic platforms (platform approach). Our convergent strategy relies on the five-step assembly of four fragments (Scheme 1). At underlying the involvement of the C7-methoxy group in stabilizing lateral contact interactions with a second tubulin unit.8 The synthesis of aldehydic fragment (±)-4a could be readily carried out in six steps (Scheme 2, eq 1). Convergent elaboration of the THP moiety was achieved through Pd-catalyzed cyclization, induced by excess trifluoroacetic acid,15 between the aldehyde derived from alcohol 5 and commercial bis-homoallylic alcohol 6. Methanalysis of the trifluoroacetate intermediate provided the racemic 7-hydroxytetrahydropyran (±)-7 in 65% yield as a single S,7-cis/S,9-cis diastereoisomer. Methylolation of the resulting alcohol occurred in high yield, leading to tetrahydropyran (±)-8, on which the equatorial position of all substituents was clearly deduced from 1H NMR analysis (Jδ5,6 = 11.5 Hz). Aldehyde (±)-4a resulted from a classical reduction/oxidation sequence on methyl ester (±)-8. This straightforward synthesis could be conducted on a multigram scale. Enantiopure aldehyde (SR,9S)-4b11 (Scheme 2, eq 2) was also selected as a valuable simplified C3−C11 fragment. Indeed, when the PelA polyoxygenated THP was replaced by the 7,8,9-trideoxy enantiopure analogue retained a hundred nanomolar range cytotoxicity.16 Preparation of enantiopure aldehyde (SR,9S)-4b was performed on a multigram scale, via intermediate (R)-9, according to the 10-step strategy developed in the course of our previous study.15

pro-Peloruside C15−C20 fragment (R)-1a7 and nor-methyl C15−C19 fragment (R)-1b were selected to implement our strategy (Scheme 3). A robust nine-step synthesis of pro-peloruside fragment (R)-1a was reported by Taylor’s team17 based on a chiral oxazolidinone-controlled alkylation to produce intermediate (R)-10 in five steps and a (Z)-selective Still−Gennari olefination. The above strategy was carried out, using an alternative Ando-type procedure18b with diaryl phosphonate 12,18c to generate the trisubstituted alkene in high yield with a unique (Z)-geometry (Scheme 3, eq 1). C15−C20 fragment (R)-1a could thus be readily synthesized on a multigram scale.

nor-Methyl fragment (R)-1b (Scheme 3, eq 2) was elaborated in a context where structural modification on the C-18 residue has no precedent in the literature. Original aldehyde (R)-1b, while retaining a structure very close to the natural side chain, could advantageously be more readily

Additionally, the impact of each oxygenated functionality along the macrolactone skeleton has not been systematically explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4b as well as dually bound PelA/Taxon to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,4a as well as dually bound PelA/Taxol to microtubules,4b providing structural information on the binding mode.
synthesized in only six steps from (S)-Roche ester. This commercially available precursor was first transformed into the corresponding TIPS-protected aldehyde (S)-13 in three steps. The latter underwent Z-selective olefination with phosphonate 12 to provide exclusively the expected (Z)-trisubstituted alkene. A reduction/oxidation sequence of the ester moiety led to the targeted aldehyde (R)-1b. This synthesis could be achieved on a multigram scale in 57% global yield over six steps.

Coupling of C15–C20 fragments 1 with TES-diazoacetone 2 (C12–C14 fragment)14 and cinnamic acid 3 (C1–C2 fragment) was then performed (Scheme 4).

Scheme 4. Coupling between C12–C14, C15–C20, and C1–C2 Fragments

At first, low-temperature LDA-induced methyl-side aldolizations were performed between aldehydes (R)-1a or (R)-1b and TES-diazoacetone 2, prepared in three steps on a multigram scale.1 Desilylation of any remaining C-protected diaoaldols was ensured by methanolysis on the crude products, providing aldols 14a (R = Et) and 14b (R = Me) as inseparable mixtures of two diastereoisomers. Absolute configurations of major (1S)-14 and minor (1R)-14 diastereoisomers were attributed on the basis of Mosher’s esters study on the diastereoisomeric mixtures (see the SI).20 Pleasingly, esterification of cinnamic acid 3 with alcohols 14a and 14b under Steglich conditions allowed clean separation of the resulting (1S)-15 and (1R)-15 diastereoisomers in both series. The cinnnamic moiety constituted in our hands a stable surrogate to the acrylic moiety, whose introduction failed whatever the conditions used (acyryloyl chloride/DMPEA or acrylic acid/DCC/DMAP). Assembly of C3–C11 pyran fragments (±)-4a and (SR, 9S)-4b with diazoketones (15S)-15a and (15S)-15b was then carried out via low-temperature, LDA-induced, diazo-side addition (Scheme 5).21 This step constituted a key feature in the establishment of our diazo strategy, as it was known to be particularly suitable for hindered tetrahydropyranic aldehydes.14 Reaction time and temperature were optimized in order to prevent simultaneous elimination of the cinnamic ester moiety (leading to the unwilled C14,C15-unsaturated, highly conjugated system). The mixture of diastereoisomers resulting from this coupling was directly subjected to a dediazotation/1,2-H migration process catalyzed by Rh2(OAc)4,14,22 leading to a diastereomeric mixture of keto-enols 16aa and 16ba and to enantiopure keto-enols 16ab and 16bb. The tautomeric structure of the sole keto-enol 16 was generated with a unique (E)-geometry (J2–3 = 15.8 Hz). The convergent diazo strategy was thus validated with the elaboration of stable macrocyclic platforms 17aa−17bb, involving 12 or 15 steps for the longest linear sequence (from a commercially available substrate), on a 180−500 mg scale.

Finally, the deprotection of the primary hydroxyl group on the lateral side chain of macrocyclic platforms 17 was achieved using Et3N·3HF24 or TBAF/AcOH,25 leading to the

Scheme 5. Coupling of C3–C11 Fragments 4 and Ring-Closing of the Macrocyclic Platforms
corresponding original peloride A unsaturated analogues 18 (Scheme 6). In vitro cytotoxic properties of these compounds were evaluated on seven tumor cell lines: Huh7 and NCI (liver), Caco2 and HCT116 (lung), PC3 (prostate), and MCF7 and MDA-MB-231 (breast). Enantiopure analogue 18ab, featuring the ethyl-substituted lateral chain, was active in a low micromolar range on two tumor cell lines (NCI: IC_{50} = 5 μM; MCF7: IC_{50} = 6 μM), while its congener with the methyl chain 18bb was less active (NCI: IC_{50} = 30 μM; MCF7: IC_{50} = 16 μM). Interestingly, the 7,8,9-trideoxy-THP moiety did not wipe out the cytotoxicity. Therefore, even though our analogues proved only slightly active, this observation is in accordance with Altmann’s team results. Analogues 18aa and 18ba, although obtained as 1:1 diastereoisomeric mixtures, were also tested, in order to gain a first-order approximation of their cytotoxic properties. Only 18aa displayed a weak activity (Caco2: IC_{50} = 15 μM).

To conclude, a function-oriented strategy toward original PeLA analogues has been developed. The convergent elaboration of stable unsaturated macrocyclic platforms with a PeLA skeleton has been achieved, involving 12 or 15 steps in the longest linear synthetic sequence. The four-fragment diazo strategy implemented allows introduction of diversity by modulating the substituents on the C3−C11 and C15−C20 fragments. The low-micromolar cytotoxicity effects of analogue 18ab is encouraging and seems to highlight the importance of the ethyl residue on the lateral chain. Further functionalization of the macrocyclic platforms is under investigation for positions C2, C3, C11, and C13 to provide a library of PeLA analogues in order to enrich the SAR studies.

Scheme 6. Deprotection of the Macroyclic Platforms and Significant in Vitro Cytotoxic Activities

**REFERENCES**


(21) Minor diastereoisomers (15R)-15a and (15R)-15b, produced in significant amounts, will constitute valuable C15–C20 fragments for the future elaboration of a library of PelA analogues in order to complete the SAR studies.


(23) Diastereoisomeric mixtures of macrocycles 17aa, 17ba, 18aa, and 18ba could not be efficiently separated by silica gel column chromatography; only partial separation was achieved.


(26) Ongoing synthetic efforts focus on the elaboration of enantiopure C3-C11 fragment 4 in order to access the corresponding stereopure macrocyclic platforms and to provide accurate biological evaluation of analogues 18aa and 18ba.

(27) All the IC50 values and the protocol used for in vitro assays of cell proliferation inhibition are described in the SI. The compounds were first tested at a unique dose of 25 μM, and IC50 was then calculated for the active ones.