Total Synthesis

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Total Synthesis of the Thiazolyl Peptide GE2270 A**

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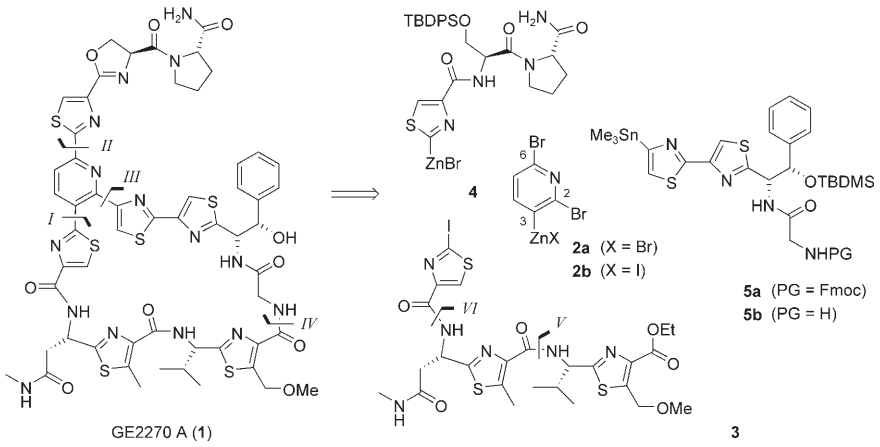
Dedicated to Professor George A. Olah on the ocassion of his 80th birthday

GE2270 A (**1**) is the prototypical member of the GE2270 thiazolyl peptides,[1] a family of antibiotics produced by *Planobispora rosea*.[2,3] The initially proposed structure[4] was revised in 1995[5] when the stereochemical configuration was shown to be derived from naturally occurring amino acids. The absolute and relative configuration of the phenylserine fragment was elucidated in 2005.[6] The structural assignment of GE2270 A has been recently confirmed by a high-resolution (1.6 Å) X-ray crystal structure analysis of the complex of GE2270 A and the bacterial elongation factor EF-Tu[7] and by a total synthesis.[8] Indeed, interest in the synthesis of the GE2270 thiazolyl peptides[8,9] is mainly related to their potent activity as inhibitors of bacterial protein biosynthesis.[7,10] Their unique mode of action at a bacteria-specific enzyme makes the GE2270 thiazolyl peptides important lead structures for the discovery of new antiinfective agents. We have therefore directed our attention to a synthetic route to this compound class, and we herein report on our total synthesis of GE2270 A.



Scheme 1. Synthetic strategy for the construction of the thiazolyl peptide GE2270 A (**1**) by regioselective cross-coupling reactions. Fmoc = 9-Fluorenylmethoxycarbonyl, PG = protecting group, TBDMS = *tert*-butyldimethylsilyl, TBDPS = *tert*-Butyldiphenylsilyl.

Our synthetic strategy (Scheme 1) relied on the consecutive introduction of three advanced subunits to a central pyridine core by regioselective cross-coupling reactions.[11] The zincated pyridine **2** was envisioned to react with the "southern" subunit **3** (Negishi cross-coupling,[12,13] step *I*), before the regioselective introduction of the "northern" subunit **4** at C6 (Negishi cross-coupling, step *II*) and of the "eastern" fragment **5** at C2 (Stille cross-coupling,[14] step *III*). The completion of the synthesis should then involve a macrolactamization (step *IV*) and protecting-group removal. Alternatively, the amide coupling (step *IV*) could be carried out prior to the last cross-coupling (step *III*), which should then serve to realize the ring closure. The latter strategy ultimately emerged as the more successful one.

The disconnection of the "southern" subunit **3** at the amide junctions leads back to three thiazole-containing fragments (Scheme 1, steps *V* and *VI*). While 2-iodothiazole-4-carboxylic acid could be readily synthesized in analogy to reported compounds,[15] the synthesis of the more complex chiral thiazoles required longer reaction sequences. For the preparation of thiazole **9** (Scheme 2) we applied the alkylation method recently reported by Deng und Taunton.[16] This strategy allowed the late introduction of the methoxymethyl moiety after the formation of the thiazole ring by Hantzsch synthesis. In this manner, instead of preparing a complex ketocarboxylate,[5] we could use the commercially available ethyl bromopyruvate. The thioamide **7** was obtained from *N*-*tert*-butoxycarbonyl(Boc)-protected valine (**6**), and the subsequent elaboration to the chiral thiazole **8** proceeded in excellent yield. Minor racemization in the alkylation of **8** (89% *ee*)[17] could be avoided by simply switching the protecting group from Boc to trityl (Tr) (>95% *ee*). Despite the protecting-group manipulations the overall yield for the synthesis of fragment **9** (55%) was more than acceptable.

The Gabriel synthesis[18] was chosen for the assembly of the asparagine-derived thiazole **13** (Scheme 3). Although a

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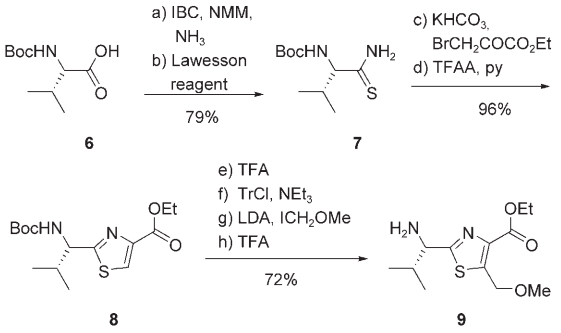
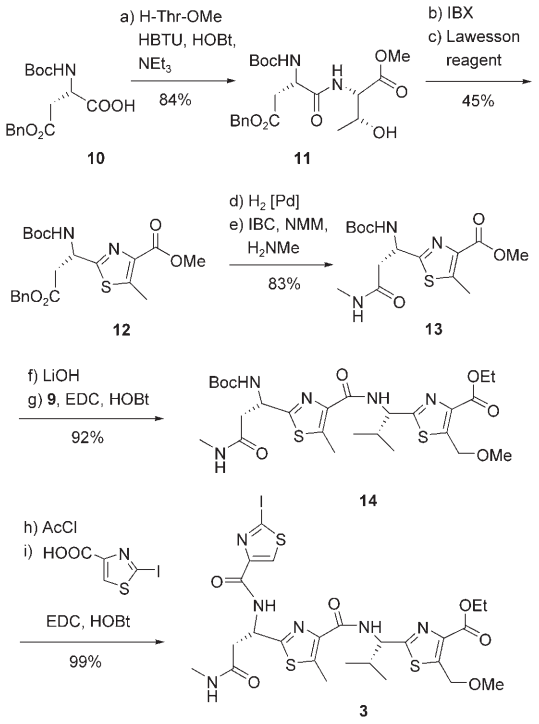


Scheme 2. Synthesis of the valine-derived thiazole **9**. Reaction conditions: a) IBC (1.05 equiv), NMM (1.1 equiv), 25% NH_3 (aq), THF, −20°C to 25°C, 16 h, 96%; b) Lawesson reagent (0.5 equiv), THF, 25°C, 16 h, 82%; c) $KHCO_3$ (8 equiv), $BrCH_2COCO_2Et$ (3 equiv), DME, −15°C, 24 h; d) TFAA (4.1 equiv), pyridine (8.8 equiv), DME, −15°C, 2 h, 96% yield over two steps; e) TFA/CH_2Cl_2 (1:5); f) TrCl (1 equiv), NEt_3 (2.6 equiv), DME, 25°C, 16 h, 98% yield over two steps; g) LDA (1.1 equiv), THF, −78°C, 1 min, then ICH_2OCH_3 (3 equiv), −78°C, 5 min, 74%; h) TFA/CH_2Cl_2 (1:5). DME = dimethoxyethane, IBC = isobutyl chloroformate, LDA = lithium diisopropylamide, NMM = *N*-methylmorpholine, py = pyridine, TFAA = trifluoroacetic anhydride, TFA = trifluoroacetic acid, TrCl = trityl chloride,.

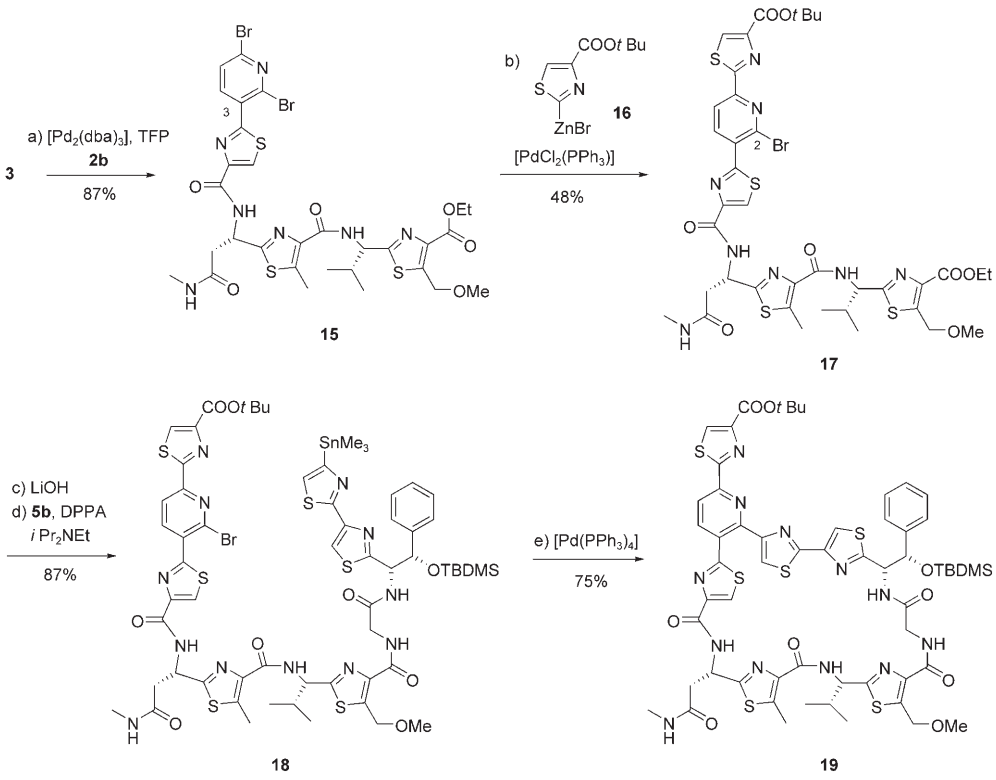


Scheme 3. Synthesis of the asparagine-derived thiazole **13** and coupling to the "southern" fragment **3**. Reaction conditions: a) H-Thr-OMe (1.2 equiv), HBTU (1.2 equiv), HOBt (1.2 equiv), NEt_3 (3.6 equiv), DMF, −25°C to 25°C, 16 h, 86%; b) IBX (2.2 equiv), MeCN, reflux, 3.5 h; c) Lawesson reagent (1.5 equiv), THF, reflux, 5 h, 45% yield over two steps; d) H_2 (1 atm), 20% $Pd(OH)_2/C$ (7 mol%), MeOH, 60°C, 16 h; e) IBC (1 equiv), NMM (1 equiv), 40% H_2NMe (aq) (1.2 equiv), THF, −25°C to 25°C, 1 h, 83% yield over two steps; f) LiOH (3.5 equiv), MeOH, 0°C to 25°C, 16 h; g) EDC (1.2 equiv), HOBt (2 equiv), DMF, −10°C to 25°C, 16 h, 92% yield over two steps; h) AcCl (10 equiv), EtOH, 0°C to 25°C, 16 h; i) EDC (1.2 equiv), HOBt (3 equiv), DMF, −10°C to 25°C, 16 h, 99% yield over two steps. EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, H-Thr-OMe = (*S*)-threonine methyl ester, IBX = 2-iodoxybenzoic acid.

method for the preparation of precursor **11**, based on the insertion of a carbene into an N−H bond, was available,[19] we followed the more conventional route.[20] The CO−NH bond was established by the peptide coupling of the carboxylic acid **10** with threonine methyl ester. The oxidation of **11**[21] yielded the corresponding ketoamide, which was treated with Lawesson reagent[22] in order to effect the heterocyclization. The hydrogenolysis of benzyl ester **12** was subsequently carried out in the presence of Pearlman's catalyst $Pd(OH)_2/C$, and the coupling of the resulting carboxylic acid with methylamine yielded the enantiomerically pure fragment **13** (> 95% *ee*)[17] in very high yield. Saponification of **13** and coupling with fragment **9**[23] proceeded without loss of stereochemical integrity. To complete the synthesis of subunit **3**, 2-iodothiazole-4-carboxylic acid was conveniently attached in excellent yield. The order of events in the peptide coupling sequence (step *V* before *VI*) proved to be crucial to avoid a possible epimerization.

The organozinc reagent **2** was generated by reductive metalation of the corresponding halide using *N*,*N*-dimethylacetamide (DMA) as solvent.[24] Extensive optimization showed that for the success of the subsequent Negishi cross-coupling of **3** the amount of DMA had to be kept as low as possible. Since the metalation of 2,6-dibromo-3-iodopyridine[25] could be carried out in a DMA/THF mixture—instead of pure DMA—and this pyridine was more conveniently obtained[26] than 2,3,6-tribromopyridine,[27] **2b** was the reagent of choice for the first Negishi cross-coupling (step *I*, Scheme 1). The yield of product **15** was remarkably high (Scheme 4). However, the second cross-coupling entailed more serious difficulties. While the organozinc **4** could be readily obtained from the corresponding bromide, the following coupling with **15** failed in all cases, even when a considerable excess of **4** (10 equiv) was employed. We reasoned that the failure could be caused by the presence of several acidic protons in our substrate. As possible alternatives we contemplated the use of an analogue of **4** tritylated at the proline amide N atom as well as zinc reagent **16**, which could be conveniently prepared from the corresponding bromide.[28] Whereas in the first case no successful coupling was observed, the desired C−C bond formation was secured in the second case. Despite the moderate yield, the reaction proceeded with high levels of regiocontrol and the introduction of the serine–proline subunit appeared unproblematic.

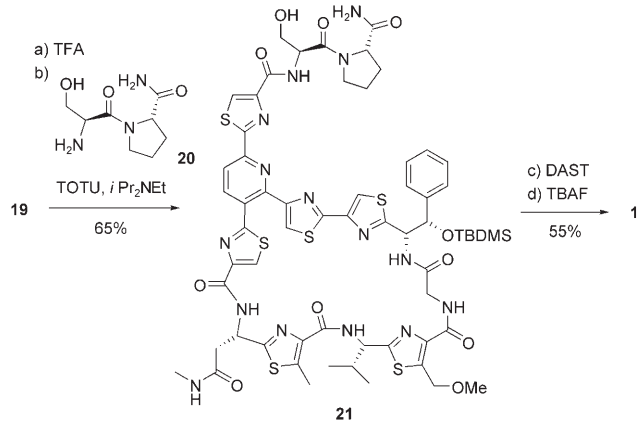
In the third consecutive cross-coupling event (step *III*) the "eastern" fragment **5a** was to be introduced at C2 in **17** by a Stille coupling. The bithiazole **5a** was synthesized in analogy to our reported procedure[29] from 2,4-dibromothiazole, TBDMS-protected (*S*)-methyl mandelate, and Fmoc-protected glycine (9 steps, 32% overall yield). The following macrocyclization (step *IV*) turned out to be troublesome. In accordance with previous experience by Nicolaou et al.,[8] the yield for the sequence involving the deprotection of the amine, saponification of the ester, and macrolactamization was in all cases below 30%. While there was no obvious



Scheme 4. Regioselective introduction of the pyridine substituents by cross-coupling and formation of macrolactam **19**. Reaction conditions: a) **2b** (3 equiv), $[Pd_2(dba)_3]$ (6 mol%), TFP (12 mol%), THF/DMA (5:1), 45°C, 16 h, 87%; b) **16** (8 equiv), $[PdCl_2(PPh_3)_2]$ (30 mol%), DMA, 45°C, 3.5 h, 48%; c) 1 M LiOH/*tert*-BuOH/THF (1:2:1), 25°C, 1 h; d) **5b** (1 equiv), DPPA (1.7 equiv), iPr_2NEt (3.4 equiv), DMF, 25°C, 16 h, 87% yield over two steps; e) $[Pd(PPh_3)_4]$ (20 mol%), toluene (1 mL μmol^{-1}), 85°C, 75%. dba = Dibenzylideneacetone, DPPA = diphenylphosphoryl azide, TFP = tri(2-furyl)phosphane.

alternative macrocyclization method in the Nicolaou synthesis, our strategy offered—as already indicated in the introduction—the possibility of reverting the coupling order. If the amide formation (step *IV*) was performed in an intermolecular fashion, the Stille coupling (step *III*) could serve to close the macrocycle. To validate this plan, removed the Fmoc group of **5a** (piperidine in DMF, 25°C, 70%), and the resulting amine **5b** was coupled with the carboxylic acid obtained from the saponification of ester **17**. To our delight, the formation of the macrocycle **19** with the Pd-catalyzed cross-coupling method proceeded in 75% yield (Scheme 4).

To complete the synthesis, the *tert*-butyl ester **19** was hydrolyzed revealing the corresponding carboxylic acid, an intermediate that had been previously converted into GE2270 A by the stepwise coupling of serine and proline amide (5 steps, 22% yield).[8] We opted for the direct coupling of the free acid with the complete dipeptide **20**, a step that proceeded in very good yield (Scheme 5). The dipeptide was conveniently prepared from L-proline in six steps and 49% overall yield.[30] Formation of the undesired diketopiperazine could be avoided when the ammonium salt of **20**, obtained upon deprotection of the *N*-Boc precursor, was mixed with **19** and TOTU prior to the addition of the base (iPr_2NEt). Finally, the formation of the oxazoline ring was achieved by exposure of **21** to an excess *N*,*N*-(diethylamino)sulfur trifluoride (DAST),[31] leading, upon cleavage of the silyl ether from the phenylserine, to GE2270 A. The obtained material was identical in all respects to the natural product.[4,5]



Scheme 5. Completion of the total synthesis of GE2270 A (**1**). Reaction conditions: a) TFA/CH_2Cl_2 (1:9), 25°C, 2 h; b) **20** (4 equiv), TOTU (1.5 equiv), iPr_2NEt (10 equiv), DMF, 25°C, 4 h, 65% yield over two steps; c) DAST (26 equiv), CH_2Cl_2, −78°C, 1 h; d) TBAF (2.5 equiv), THF, 25°C, 2 h, 55% yield over two steps. DAST = (diethylamino)sulfur trifluoride, TBAF = tetrabutylammonium fluoride, TOTU = *O*-[(ethoxycarbonyl)cyanomethyleneamino]-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate.

In summary, the thiazolyl peptide GE2270 A has been prepared in a short and convergent fashion. The synthesis proceeds in 20 steps and 4.8% overall yield (longest linear sequence) starting from the *N*-Boc-protected valine (**6**). Our strategy allows the facile modification of all building blocks and hence should be applicable to the preparation of analogous thiazolyl peptides.

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