Natural Products

Synthesis of the Heterocyclic Core of the GE2270 Antibiotics and Structure Elucidation of a Major Degradation Product**

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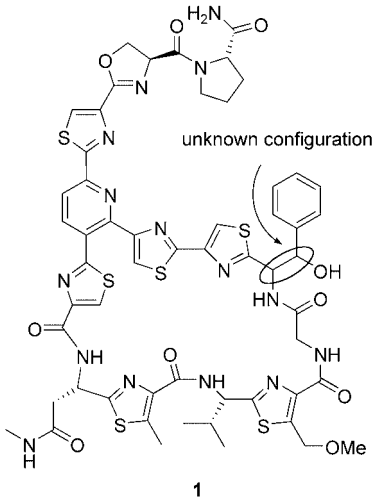
The thiazolyl peptide GE2270A (**1**) was isolated in 1991 from *Planobispora rosea* ATCC53733.[1] The compound, like many other closely related GE2270 thiazolyl peptides,[2] shows



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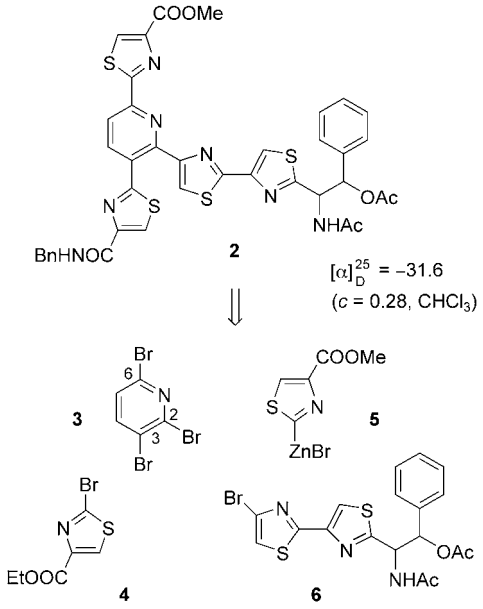
antibiotic activity by inhibiting the bacterial elongation factor (EF) Tu (GE2270A: $IC_{50} = 5$ nM).[3] EF-Tu is a protein in bacteria cells that catalyzes the binding of aminoacyl-tRNA to the A site of the ribosome.[4] In eukaryotes this function is carried out by the elongation factor EF-1 alpha, which is not inhibited by GE2270A.[5] The remarkable antibiotic activity and the demanding molecular architecture of the GE2270 thiazolyl peptides make them interesting synthetic targets.[6–8] A total synthesis of these compounds has not yet been reported.

The structure of GE2270A was established in 1991[1b] but corrected in 1995.[9] The configurations of four of the six stereogenic centers could be elucidated by degradation reactions and by structure comparison with synthetic material.[9] Only the configuration of the 1,2-amino alcohol in the eastern part of the molecule is not known. Herein we report a concise synthetic approach to the core structure of the GE2270 thiazolyl peptides and the synthesis of a degradation product that proves the absolute configuration of GE2270A.

The degradation product **2** is depicted in Scheme 1. It was obtained from GE2270A by Tavecchia et al. after hydrolysis with formic acid, treatment with Na_2CO_3/MeOH, aminolysis with benzylamine, and subsequent acetylation.[9] The compound was characterized by UV/Vis, IR, and ^{1}H NMR spectroscopy as well as by its specific rotation (Scheme 1).



Scheme 1. Retrosynthetic disconnection of the GE2270A degradation product **2** into the fragments **3**–**6**.

According to our strategy the central 2,3,6-trisubstituted pyridine moiety of the GE2270 thiazolyl peptides should be synthesized from easily accessible 2,3,6-tribromopyridine (**3**)[10] by three consecutive C–C bond-formation reactions. Pyridine **2** was used as a model system to establish our plan for the construction of the GE2270 core. In the present case we hoped to address the 3-position of the pyridine by employing a regioselective bromine–lithium exchange reaction.[11] The transmetalation step should be followed by a cross-coupling with a 2-bromothiazole (e.g. **4**). In subsequent cross-coupling reactions, positions 2 and 6 of the pyridine ring should be attacked nucleophilically.[12,13] It was our opinion that the more easily accessible position 6 would be prone to regioselective substitution by a suitable zinc reagent (e.g. **5**). The final cross-coupling reaction should be performed with a zinc, tin, or boron reagent prepared from bromide **6**.

To elucidate the structure of the degradation product **2** it was necessary to use both possible diastereomeric bromides *threo*- and *erythro*-**6** in enantiomerically pure form. We started with the bithiazoles *threo*-(*S*,*R*)-**7** (**7a**) and *erythro*-(*R*,*R*)-**7** (**7b**), which were synthesized from enantiomerically pure (*R*)-mandelonitrile (Scheme 2). Bithiazole *erythro*-(*R*,*R*)-**7** (**7b**) was generated from the corresponding *erythro* amino alcohol in full analogy to the procedure used for the synthesis of the known compound **7a**.[14] Cleavage of the *tert*-butyldimethylsilyl (TBDMS) protecting group with tetrabu-

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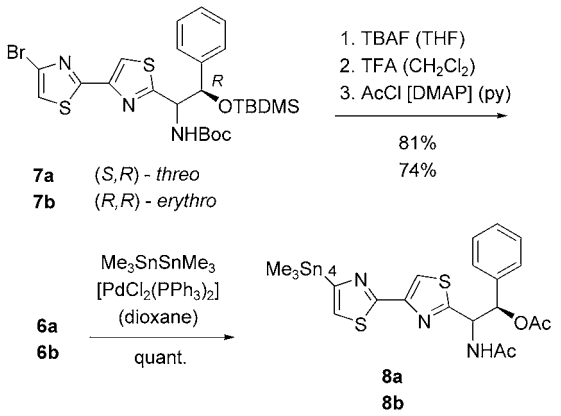
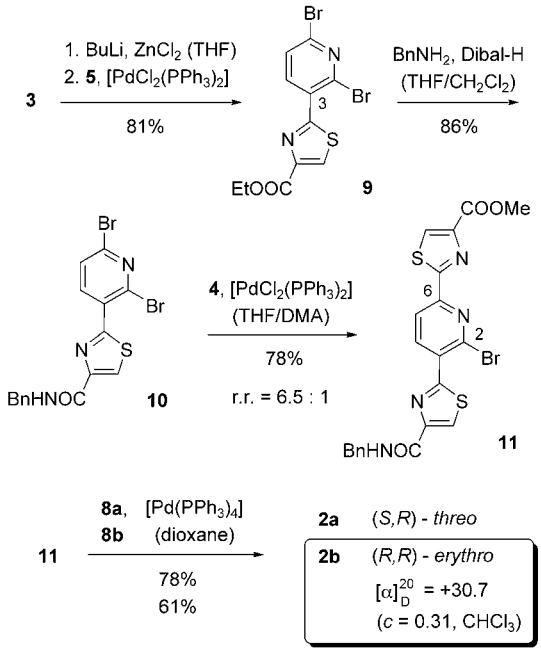


Scheme 2. Synthesis of the diastereomeric stannylated bithiazoles **8a** and **8b** from the precursors **7a** and **7b**.

tylammonium fluoride (TBAF) and removal of the *tert*-butoxycarbonyl (Boc) protecting group with trifluoroacetic acid (TFA) furnished the free amino alcohol, which was then doubly acetylated. The overall yields of these three steps for both diacetates **6a** (81%) and **6b** (74%) were satisfactory. The introduction of a metal in the 4-position of the bithiazole was best achieved by Pd-catalyzed stannylation with hexamethylditin at 80°C.[8a] Attempts to zincate after bromine–lithium exchange at the 4-position were unsuccessful. The prepared stannanes **8a** and **8b** were used directly in the next reaction.

As we had hoped, tribromopyridine **3** was converted regioselectively into the 3-lithium compound and transmetalated to the zinc compound, which underwent a Negishi cross-coupling with the ethyl ester **4**[15] (Scheme 3). This reaction



Scheme 3. Synthesis of the trisubstituted pyridines **2a** and **2b** from 2,3,6-tribromopyridine (**3**) and structure comparison with the known degradation product, which is assigned the structure *ent*-**2b**.

proceeded more smoothly than the cross-coupling with the analogous 2-bromothiazole *N*-benzylamide which was also attempted. In an additional step the amide was generated in good yield by treating ester **9** with *N*-benzylamine/diisobutylaluminum hydride (Dibal-H).[16] The next cross-coupling reaction was preceded by reductive metalation of methyl 2-bromothiazole-4-carboxylate with zinc.[17] The resulting zinc compound reacted with 2,6-dibromopyridine **10** in a Negishi cross-coupling. The regioselectivity was not completely perfect, and besides the desired 6-substituted 2-bromopyridine **11** the 2-substituted 6-bromopyridine was formed. The regioisomeric ratio (r.r.) was 6.5:1. It was necessary to apply an excess of the zinc compound to achieve a quantitative turnover. When we used 1.3, 1.8, and 2.2 equivalents of the zinc regent, the yield of **11** increased under otherwise identical conditions from 41% to 52% to 62%. In the last case 20% of the starting material could be recovered. Finally, the inseparable mixture of regioisomers underwent a Stille cross-coupling with the stannanes **8a** and **8b**.[18] The reaction proceeded well without complications at 80°C in dioxane. The mixture of regioisomers was separated by semipreparative HPLC.[18]

The ^{1}H NMR spectra of the degradation product[9] and of the *erythro* isomer **2b**[18] match perfectly, whereas the spectra of the *threo* isomer **2a** exhibit significant differences. The differences are found mainly at the spin system of the N,O-diacetylated amino alcohol. The chemical shift of the C*H*(OAc)-proton of the degradation product was reported as $\delta = 6.20$ ppm and its coupling constant as $^3J = 8.0$ Hz[9] (**2a**: 6.32, $^3J = 5.2$ Hz; **2b**: 6.19, $^3J = 8.0$ Hz). The C*H*(NHAc) proton gives rise to a virtual triplet at $\delta = 5.60$ ppm and $^3J \cong 8.1$ Hz (**2a**: 5.66, dd, $^3J = 8.8$, 5.2 Hz; **2b**: 5.58, virt. t, $^3J \cong 8.4$ Hz). The synthetic (*R*,*R*) enantiomer **2b** exhibited a positive specific rotation (Scheme 3, $[\alpha]_D^{20} = +30.7$ ($c = 0.31$, $CHCl_3$)), and consequently a (*S*,*S*) configuration could be assigned to the degradation product and also to the thiazolyl peptides GE2270. The absolute values of the specific rotation for **2b** (Scheme 3) and for the degradation product *ent*-**2b** (Scheme 1) are nearly identical, whereas for **2a** a specific rotation of $[\alpha]_D^{20} = -2.9$ ($c = 0.56$, $CHCl_3$) was determined.

The construction of the core fragment of the thiazolyl peptides GE2270 described herein should also be applicable to the synthesis of the natural product. The cross-coupling reactions allow a high degree of functionality within the different reaction partners and should facilitate the linkage of more complex building blocks. Studies in this direction and further optimization of the cross-coupling chemistry are currently being carried out.

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[18] A solution of bromopyridine **11** (27.4 mg, 53.0 μmol; r.r. = 6.5:1), stannane **8b** (35.2 mg, 64.0 μmol), and tetrakis(triphenylphosphine)palladium(0) (6.1 mg, 10 mol %) in 1.5 mL of dioxane was stirred under an argon atmosphere for 16 h at 80 °C. Saturated ammonium chloride solution (15 mL) was added to the dark brown suspension, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and filtered. A small portion of silica gel was added, and the solvent was removed in vacuo. The residue was purified by flash chromatography (silica gel 60, 2 × 18 cm, solvent: ethyl acetate) and 26.5 mg (61 %) of the desired product (r.r. = 6.5:1) was obtained. To separate the regioisomers a portion of the product was purified by semipreparative HPLC (YMC, ODS-A, 250 × 20; MeCN/H_2O 20:80 → 100:0 in 30 min; flow = 15.0 mL min^{-1}). The desired product **2b** was isolated as a colorless solid. $R_f = 0.43$ (EtOAc); $[\alpha]_D^{20} = +30.7$ ($c = 0.31$, $CHCl_3$); UV (MeCN/H_2O = 89/20): $\lambda_{max} = 302$ nm; ^{1}H NMR ([D$_6$]DMSO, 360 MHz): $\delta = 1.75$ (s, 3H), 2.00 (s, 3H), 3.90 (s, 3H), 4.46 (d, $^3J = 6.1$ Hz, 2H) 5.58 (virt. t, $^3J \cong 8.4$ Hz, 1H), 6.19 (d, $^3J = 8.0$ Hz, 1H), 7.21–7.40 (m, 10H), 7.90 (s, 1H), 8.29 (s, 1H), 8.34 (d, $^3J = 8.2$ Hz, 1H), 8.37 (s, 1H), 8.75 (d, $^3J = 8.2$ Hz, 1H), 8.75 (s, 1H), 8.84 (d, $^3J = 8.6$ Hz , 1H), 9.10 ppm (t, $^3J = 6.2$ Hz, 1H); ^{13}C NMR ([D$_6$]DMSO, 90 MHz): $\delta = 21.0$ (q), 22.5 (q), 42.4 (t), 52.4 (q), 54.4 (d), 74.8 (d), 118.1 (d), 119.2 (d), 123.3 (d), 126.9 (d), 127.3 (d), 127.4 (d), 128.4 (d), 128.6 (d), 129.6 (s), 132.5 (d), 137.1 (s), 139.7 (s), 140.1 (d), 147.4 (s), 147.5 (s), 149.8 (s), 150.1 (s), 151.0 (s), 152.8 (s), 160.6 (s), 161.3 (s), 162.1 (s), 163.6 (s), 167.9 (s), 169.2 (s), 169.3 (s), 170.5 ppm (s), two aromatic CH signals overlap; HRMS (FAB): $C_{39}H_{32}N_7O_6S_4$ [$(M+H)^+$] calcd: 822.1297, found: 822.1289.

