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Total Synthesis of Pseudotrienic Acid B: A Bioactive Metabolite from *Pseudomonas* sp. MF 381-IODS**

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In memory of Pierre Potier

Pseudotrienic acids A and B are two bioactive metabolites with antimicrobial activity that have been recently isolated from *Pseudomonas* sp. MF 381-IODS.[1] Their structures were established by ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectroscopy and by ESI mass spectrometry. Each of the pseudotrienic acids A and B was isolated as a 1:1 mixture of epimers at C20, but the absolute configurations at C11 and C12 were well defined as *S* and *R*, respectively. The molecular architecture of these compounds is further characterized by a *E*,*E*,*E* trienic conjugated acid (C1–C7), two amide bonds, and a trisubstituted conjugated *E*,*E* diene (C16–C19).

Both pseudotrienic acids A and B inhibit growth of *Staphylococcus aureus* (minimum inhibitory concentration (MIC) of 70 μg mL^{-1}) and *Pseudomonas syringae* pv. *syringae* (MIC of 70 μg mL^{-1}). Moreover, Pohanka et al.[1] observed that pseudotrienic acids A and B were prone to macrolactonization in an acidic environment and, therefore, could be transformed into two naturally occurring 19-membered-ring lactones, FR252922 and FR252921, respectively, by treatment with trifluoroacetic acid (TFA) (Scheme 1). These two macrolactones, isolated in 2003 by Fujine et al.[2] from the culture broth of *Pseudomonas fluorescens* no. 408813, showed immunosuppressive activity against murine splenocyte proliferation stimulated with a lipopolysaccharide (LPS) or anti-CD3 in vitro.[2] Interestingly, their site and mechanism of action seem to be distinct from those of FK-506 and cyclosporin A (CsA).[2] To date, the configuration of the three stereocenters in FR252922 and FR252921 has not been established. However, it was suggested that the pseudotrienic acids A and B are derived from ring opening of the two macrolactones FR252922 and FR252921, respectively, by a nucleophilic attack of H_2O at C20 according to a nonstereospecific S_N'' substitution, thus leading to a mixture of epimers at C20 (Scheme 1).[1] However, since the biosynthesis of polyketides of this type is usually stereospecific, the possi-

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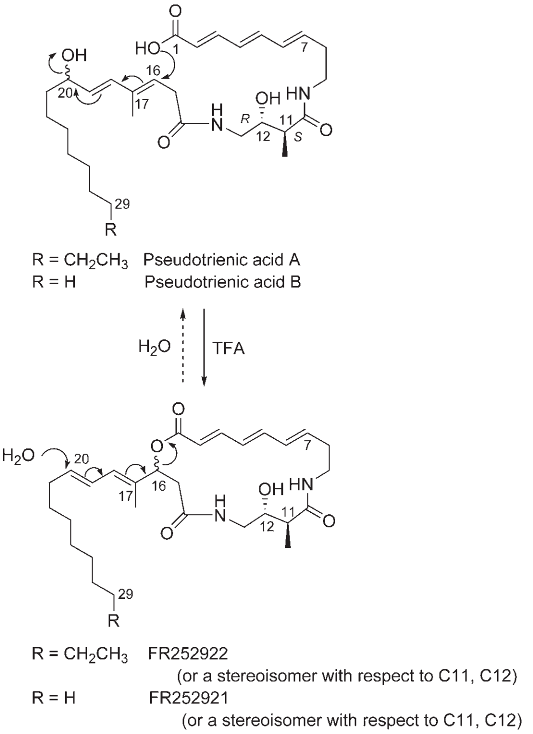
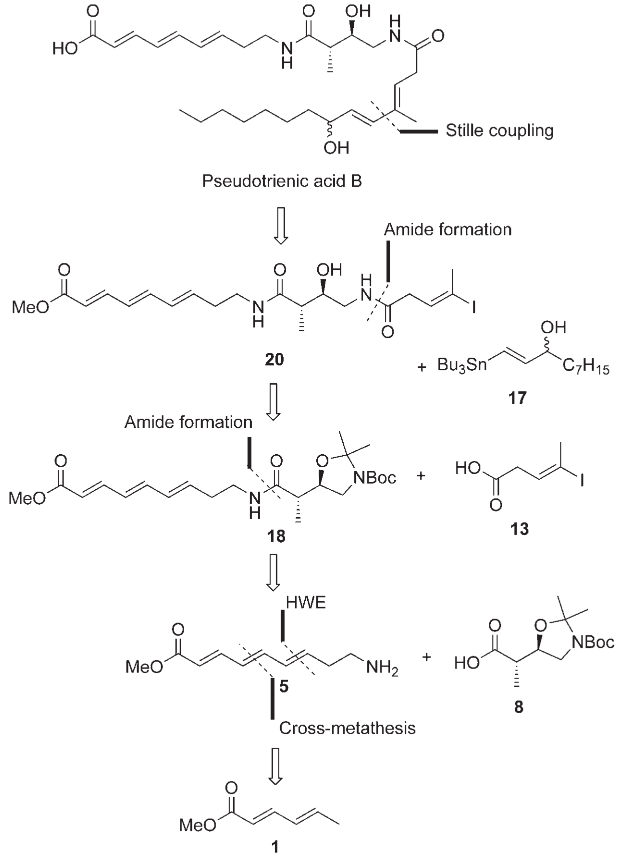


Scheme 1. Lactone formation of FR252921 and FR252922 under acidic conditions.

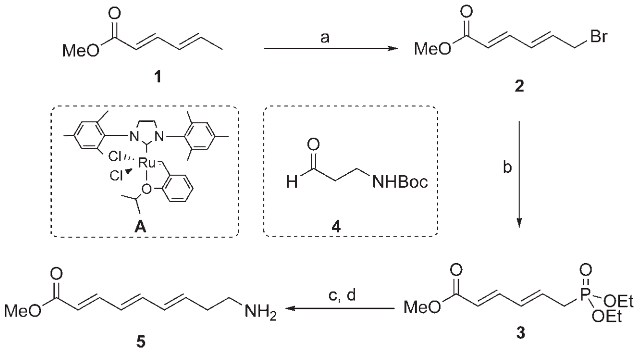
bility that an epimerization took place during the isolation process of the natural product cannot be ruled out.

Based on these observations, it seems reasonable to assume that FR252922 and FR252921 possess the absolute configuration *S* at C11 and *R* at C12, but the configuration at C16 in the two latter compounds remains to be established. One can suggest that if both epimers at C20 of pseudotrienic acid B were prepared, an S_N2'' reaction carried out in an *anti* stereospecific fashion could transfer the configuration from C20 in pseudotrienic acid B to C16 in FR252921. This should then lead to a correct assignment of this stereogenic center in FR252921. The inherent structure of pseudotrienic acids A and B, as well as the biomimetic transformation, makes these antimicrobial agents interesting targets for total synthesis. Herein, we report the first highly convergent total synthesis of pseudotrienic acid B as a mixture of epimers at C20.

The strategy employed to synthesize pseudotrienic acid B is depicted in Scheme 2. It relied on a Stille coupling between the vinyl iodide **20** and the vinyl stannane **17** to generate the *E*,*E* diene. The alkenyl iodide **20** could be obtained by combining two key fragments, carboxylic acid **13** and trienic protected amine **18**, through amide formation. A previous amide formation between the carboxylic acid **8** and the trienic amine **5** would install the second amide bond. Finally, a cross-metathesis/HWE sequence was envisaged for the initial synthesis of the trienic ester **5** from commercially available methyl sorbate (**1**).



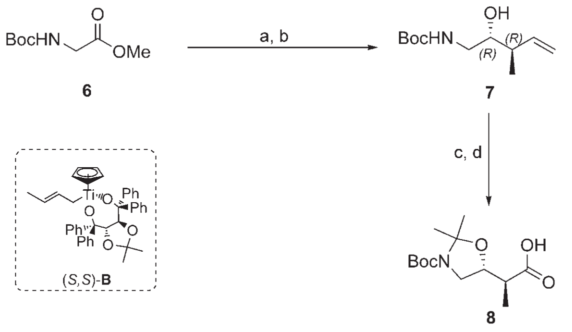
Scheme 2. Retrosynthetic analysis of pseudotrienic acid B. Boc = *tert*-butyloxycarbonyl, HWE = Horner–Wadsworth–Emmons reaction.



Scheme 3. Synthesis of amino trienoate **5**. Reagents and conditions: a) allyl bromide (5 equiv), **A** (2 mol%), CH_2Cl_2, RT, 48 h, 48%; b) $P(OEt)_3$, toluene, reflux, 1 h, 99%; c) LDA (1.4 equiv), aldehyde **4**, THF, −78 °C for 15 min then 0 °C for 30 min; d) TFA/CH_2Cl_2 (1:5), 0 °C→RT, then Na_2CO_3, 52% (2 steps). LDA = lithium diisopropylamide, THF = tetrahydrofuran.

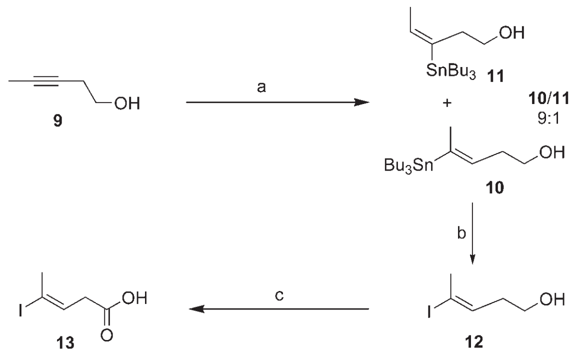
Scheme 3 delineates the synthesis of the amino trienoate **5**, which commenced with methyl sorbate (**1**) and proceeded through cross-metathesis and a Horner–Wadsworth–Emmons reaction as the key steps. Thus, methyl sorbate (**1**) was first treated with allyl bromide (5 equiv) in CH_2Cl_2 at room temperature for 48 h, in the presence of the Grubbs–Hoveyda catalyst[3] (**A**, 2 mol%), to produce **2** in 48% yield with good stereoselectivity (*E*,*E*/*E*,*Z* > 95:5).[4] Subsequent phosphonation of the dienic allylic bromide **2** under Michaelis–Arbuzov conditions led to the formation of diethylphosphonate **3** in 99% yield.[5] It is worth noting that a cross-metathesis reaction between methyl sorbate and diethyl allylphosphonate also furnishes **3** with a similar yield but, with this procedure, the purification of **3** was complicated by difficulties in the removal of ruthenium byproducts from this highly polar compound. Horner–Wadsworth–Emmons olefination between phosphonate **3** and the *N*-Boc-protected amino aldehyde **4** was accomplished in the presence of LDA[6] and resulted, after TFA-mediated deprotection, in the formation of the free trienic amine **5** in 52 % yield. Thus, the trienic ester **5** was synthesized efficiently in four steps from methyl sorbate (**1**) in approximately 25 % overall yield.

The synthesis of the amino acid **8**, which was to be coupled with **5** to produce the C1–C14 fragment of pseudotrienic acid B, was achieved by starting from the *N*-Boc-protected glycine methyl ester **6** (Scheme 4). In order to control the configuration of the two stereogenic centers C11 and C12 in pseudotrienic acid B, the amino ester **6** was transformed into the corresponding aldehyde by reduction with DIBAL-H,[7] and then treated directly with the highly face-selective crotyltitanium complex (*S*,*S*)-**B**,[8] which gave rise to the homoallylic amino alcohol **7** in 57 % yield with high diastereoselectivity (d.r. > 95:5) and enantioselectivity (> 95 % *ee*).[9] Subsequent protection of the obtained amino alcohol as the *N*,*O*-acetonide by using 2,2-DMP followed by ruthenium-catalyzed oxidative cleavage of the terminal olefin under Sharpless conditions[10] led to the desired carboxylic acid **8** with an overall yield of 75 % for the two steps (Scheme 4).



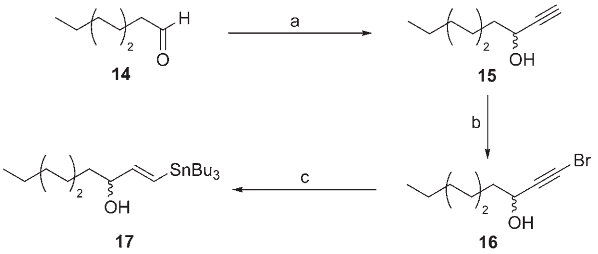
Scheme 4. Synthesis of carboxylic acid **8**. Reagents and conditions: a) DIBAL-H in toluene (1.1 equiv), CH_2Cl_2, −78 °C, 2 h; b) (*S*,*S*)-**B** (1.3 equiv), Et_2O, −78 °C, 12 h, 57% (2 steps); c) 2,2-DMP, *p*TsOH (0.1 equiv), acetone, RT, 5 min; d) $RuCl_3$ (0.05 equiv), $NaIO_4$ (4.0 equiv), $CCl_4/CH_3CN/H_2O$ (2:2:3), RT, 12 h, 75% (2 steps). DIBAL-H = diisobutylaluminum hydride, 2,2-DMP = 2,2-dimethoxypropane, *p*TsOH = toluene-4-sulfonic acid.

The *E* vinyl iodide **13**, which corresponds to the C16–C19 fragment of pseudotrienic acid B, was synthesized in three steps from the commercially available pent-3-yn-1-ol (**9**) as outlined in Scheme 5. Regio- and stereoselective stannylcupration with the mixed higher-order cuprate reagent $[(Bu_3Sn)BuCu(CN)Li_2]$[11] in the presence of MeOH afforded the two known vinyl stannanes **10** and **11** in a 9:1 ratio with a combined yield of 74 %. These two regioisomers were separated by flash chromatography on silica gel and the desired *E* vinyl stannane **10** was subjected to iododestannylation to give the known vinyl iodide **12** in very high yield (94 %).[12] Oxidation with Jones' reagent afforded the desired carboxylic acid **13** in 92 % yield.



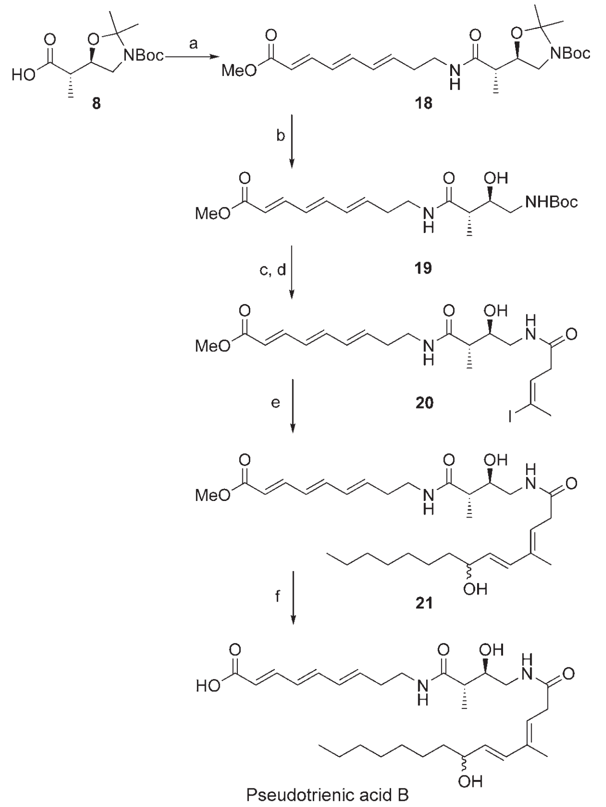
Scheme 5. Synthesis of vinyl iodide **13**. Reagents and conditions: a) CuCN (4.0 equiv), *n*BuLi (8.0 equiv), nBu_3SnH (8.0 equiv), MeOH (0.2 equiv), THF, −40 °C, 12 h, 74 %; b) I_2, Et_2O, 0 °C→RT, 94 %; c) $CrO_3–H_2SO_4$ (3.1 equiv), acetone, 0 °C, 30 min, 92 %.

The last fragment, the vinyl stannane **17** (C20–C29 fragment), was prepared from the commercially available octanal (**14**) (Scheme 6). After addition of ethynyl magnesium bromide, the resulting propargylic alcohol **15** (81 % yield)[13,14] was treated with NBS in the presence of $AgNO_3$, thus leading to the bromoalkyne **16** in 93 % yield. Regio- and stereocontrolled (> 95 % *E* isomer) Pd-mediated hydrostannylation[15] of bromoalkyne **16** with nBu_3SnH in the presence of 5 mol % of $[PdCl_2(PPh_3)_2]$ afforded the desired alkenyl stannane **17** in 70 % yield (Scheme 6).



Scheme 6. Synthesis of vinyl stannane **17**. Reagents and conditions: a) HC≡CMgBr (2.0 equiv), THF, 0 °C→RT, 12 h, 81 %; b) NBS (1.1 equiv), $AgNO_3$ (0.1 equiv), acetone, 12 h, RT, 93 %; c) nBu_3SnH (4.0 equiv), $[PdCl_2(PPh_3)_2]$ (0.05 equiv), THF, 0 °C, 4 h, 70%. NBS = *N*-bromosuccinimide.

With the four fragments in hand, the coupling reactions were performed as shown in Scheme 7. First, the carboxylic acid **8** was coupled with the amino trienic ester **5** by using HOBt, HBTU, and NMM in acetonitrile and amide **18** was isolated in 96 % yield (Table 1). In order to obtain the C1–C19 fragment of pseudotrienic acid B, compound **18** was treated with *p*TsOH in MeOH to afford the *N*-Boc-protected amino alcohol **19** in 82 % yield.[16] After removal of the *tert*-butyloxycarbonyl group, the resulting amino alcohol was coupled with the carboxylic acid **13** to produce compound **20**, the C1–C19 fragment of pseudotrienic acid B (Table 1).



Scheme 7. Completion of the total synthesis of pseudotrienic acid B. Reagents and conditions: a) **5** (1.2 equiv), HOBT (1.2 equiv), HBTU (1.2 equiv), NMM (3.0 equiv), CH_3CN, 0 °C→RT, 6 h, 96 %; b) *p*TsOH (0.5 equiv), MeOH, 4 h, RT, 82 %; c) TFA/CH_2Cl_2 (1:5), 0 °C→RT, 45 min; d) **13** (1.1 equiv), HOBT (1.1 equiv), HBTU (1.1 equiv), NMM (3.0 equiv), CH_3CN, 0 °C→RT, 12 h, 78 % (2 steps); e) **17** (1.5 equiv), $[PdCl_2(MeCN)_2]$ (0.05 equiv), DMF degassed with Ar, RT, 12 h, 51 %; f) LiOH (30 equiv), MeOH/THF/H_2O (2:2:1), RT, 12 h, 75 %. HOBT = *N*-hydroxybenzotriazole, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NMM = *N*-methylmorpholine, DMF = *N*,*N*-dimethylformamide, TFA = trifluoroacetic acid.

Table 1: Selected analytical data for compounds **18**, **20**, and **21**.

18: $[\alpha]_D^{20} = -4.50$ ($c = 1.36$, MeOH); IR (film): $\tilde{\nu}_{max} = 3424$, 3338, 2978, 2938, 1693, 1658, 1616, 1542, 1392, 1367, 1253, 1140, 1007, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.29$ (dd, $J = 15.2$, 11.2 Hz, 1 H), 6.51 (dd, $J = 14.8$, 10.8 Hz, 1 H), 6.30–6.15 (m, 2 H and NH), 5.87 (m, 1 H), 5.87 (d, $J = 15.2$ Hz, 1 H), 4.09 (m, 1 H), 3.75 (s, 3 H), 3.64 (br s, 1 H), 3.37 (m, 2 H), 3.11 (t, $J = 9.9$ Hz, 1 H), 2.37 (m, 3 H), 1.56 (br s, 3 H), 1.51 (br s, 3 H), 1.47 (s, 9 H), 1.14 ppm (br s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 173.2$ (s), 167.5 (s), 152.2 and 151.8 (s), 144.6 (d), 140.4 (d), 136.1 (d), 131.9 (d), 128.8 (d), 120.4 (d), 94.0 and 93.5 (s), 80.4 and 79.8 (s), 75.0 (d), 51.5 (q), 49.6 and 49.5 (t), 44.2 and 44.0 (d), 38.5 (t), 33.1 (t), 28.3 (3 q), 27.3, 26.2, 25.2, and 24.4 (2 q), 13.4 ppm (q); HRMS (CI^+, NH_3): calcd for $C_{23}H_{37}O_6N_2$ $[M+H^+]$: 437.2652; found: 437.2643.

20: $[\alpha]_D^{20} = +6.93$ ($c = 0.88$, $CHCl_3$); IR (film): $\tilde{\nu}_{max} = 3293$, 2930, 1711, 1639, 1616, 1541, 1433, 1263, 1233, 1136, 1004, 732, 618 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.28$ (dd, $J = 15.3$, 11.3 Hz, 1 H), 6.78 (br m, NH, 1 H), 6.57 (br m, NH, 1 H), 6.52 (dd, $J = 14.9$, 10.8 Hz, 1 H), 6.31 (br t, $J = 7.5$ Hz, 1 H), 6.25 (dd, $J = 14.9$, 11.3 Hz, 1 H), 6.20 (dd, $J = 15.4$, 10.8 Hz, 1 H), 5.87 (d, $J = 15.3$ Hz, 1 H), 5.87 (m, 1 H), 4.61 (m, 1 H), 3.74 (s, 3 H), 3.66 (br m, OH, 1 H), 3.51 (ddd, $J = 11.5$, 6.6, 4.2 Hz, 1 H), 3.34 (m, 2 H), 3.12 (ddd, $J = 11.5$, 7.5, 4.9 Hz, 1 H), 2.97 (d, $J = 7.5$ Hz, 2 H), 2.40 (s, 3 H), 2.40–2.31 (m, 3 H), 1.24 ppm (d, $J = 7.0$ Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 175.8$ (s), 170.2 (s), 167.5 (s), 144.7 (d) 140.4 (d), 135.7 (d), 132.8 (d), 132.1 (d), 128.9 (d), 120.3 (d), 97.8 (s), 73.2 (d), 51.6 (q), 44.4 (t), 43.2 (d), 38.5 (t), 37.7 (t), 33.0 (t), 27.9 (q), 15.7 ppm (q); HRMS (CI^+, NH_3): calcd for $C_{20}H_{30}O_5N_2I$ $[M+H^+]$: 505.1199; found: 505.1195.

21: $[\alpha]_D^{20} = +7.37$ ($c = 0.38$, $CHCl_3$); IR (film): $\tilde{\nu}_{max} = 3308$, 2956, 2927, 2856, 1720, 1646, 1619, 1546, 1459, 1435, 1271, 1137, 1074, 1007, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.22$ (dd, $J = 15.2$, 11.2 Hz, 1 H), 6.45 (dd, $J = 14.7$, 10.7 Hz, 1 H), 6.29 (m, NH, 1 H), 6.20 (d, $J = 15.7$ Hz, 1 H), 6.20–6.04 (m, 2 H and NH), 5.81 (m, 1 H), 5.80 (d, $J = 15.2$ Hz, 1 H), 5.62 (dd, $J = 15.6$, 6.8 Hz, 1 H), 5.53 (t, $J = 7.6$ Hz, 1 H), 4.33 (d, $J = 5.9$ Hz, OH, 1 H), 4.09 (m, 1 H), 3.68 (s, 3 H), 3.55 (br m, 1 H), 3.45 (m, 1 H), 3.29 (m, 2 H), 3.03 (m, 3 H), 2.31 (q, $J = 6.8$ Hz, 2 H), 2.22 (m, 1 H), 1.70 (s, 3 H), 1.55–1.40 (m, 2 H), 1.30–1.15 (m, 10 H and OH), 1.18 (d, $J = 7.6$ Hz, 3 H), 0.81 ppm (t, $J = 6.7$ Hz, 3 H); ^3C NMR (75 MHz, $CDCl_3$): $\delta = 175.6$ (s), 172.0 (s), 167.6 (s), 144.7 (d), 140.4 (d), 137.6 (s), 135.7 (d), 133.9 (d), 132.2 (d), 132.0 (d), 128.9 (d), 123.2 (d), 120.4 (d), 73.5 (d), 72.9 (d), 51.6 (q), 44.4 (t), 43.2 (d), 38.5 (t), 37.5 (t), 36.2 (t), 33.0 (t), 31.8 (t), 29.6 (t), 29.3 (t), 25.5 (t), 22.7 (t), 15.7 (q), 14.1 (q), 12.8 ppm (q); HRMS (CI^+, NH_3): calcd for $C_{30}H_{49}O_6N_2$ $[M+H^+]$: 533.3591; found: 533.3597.

The synthesis of pseudotrienic acid B was completed by performing a $[PdCl_2(MeCN)_2]$-catalyzed Stille cross-coupling reaction in DMF[17] between vinyl iodide **20** and vinyl stannane **17** with a nonprotected allylic alcohol, thus producing the core structure of pseudotrienic acid B in 51 % yield as a mixture of two diastereoisomers, epimeric at C20 (compound **21**) (Table 1). It is noteworthy that only one set of NMR signals is observed, a fact suggesting that these two diastereoisomers cannot be distinguished by NMR spectroscopy since they give an identical spectrum. Finally, a simple saponification of the methyl ester **21** with LiOH afforded the expected pseudotrienic acid B in 75 % yield.[18] The spectroscopic data for synthetic pseudotrienic acid B were in agreement with those reported in the literature for the natural product.[1]

In conclusion, a concise, efficient, and highly convergent stereoselective synthesis of the bioactive natural product pseudotrienic acid B has been achieved, with a longest linear sequence of 10 steps from methyl sorbate (**1**) (5.8 % overall yield). Synthetic highlights include a crotyltitanation reaction to control the stereogenic centers at C11 and C20, a cross-metathesis reaction to synthesize the triene moiety, and a palladium-catalyzed Stille cross-coupling reaction to complete the assembly of the carbon framework of pseudotrienic acid B. This rapid and flexible synthetic approach will allow access to a wide variety of analogues for biological evaluation. The possibility of assigning the configuration at C16 in FR252921 by the stereoselective synthesis of each of the two epimers of pseudotrienic acid B is currently under investigation.

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[18] Only one set of NMR signals is observed for the mixture of epimers at C20.