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NOVEL DERIVATIVES OF PSEUDOMONIC ACID

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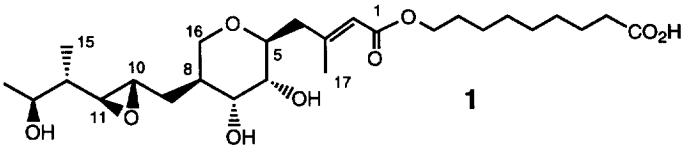
Abstract: The synthesis of a new series of pseudomonic acid analogs is described. The synthetic approach is based on replacement of the C9-C14 sidechain of the parent molecule with a modified amino substituent. The most potent member of the compound series contains a 3-chloropropyl sulfonamide moiety at the C-8 position.

The pseudomonic acids, produced by *Pseudomonas fluorescens*,[1-5] are antibiotics that act via the inhibition of isoleucyl tRNA synthetase.[6-9] The major metabolite, pseudomonic acid A (PMA) **1**,[1,2] is a potent antibiotic against a variety of Gram positive organisms,[10] and is currently used as a topical and intranasal antibiotic. Related compounds have also been found to be produced by marine bacterial species.[11,12] PMA has generated much interest in the fields of chemistry and biology due to both its novel chemical structure and its mode of action. A number of reports have appeared in connection with the total synthesis of PMA and synthesis of analogs in SAR studies.[13-17]



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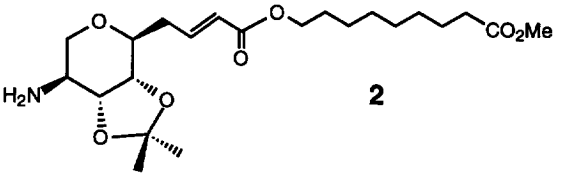
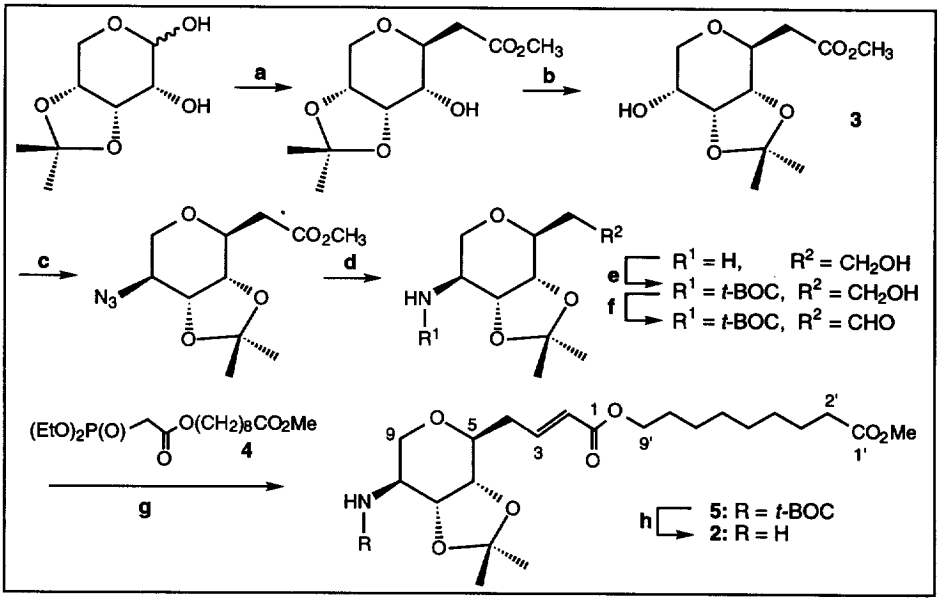
In connection with our on-going program to discover novel antibiotics that inhibit the function of aminoacyl tRNA synthetases, as well as a general interest in obtaining details concerning the mode of action of PMA, an effort was undertaken to prepare PMA analogs bearing replacements for the C9-C14 "left-hand" sidechain. It has been proposed that the left-hand sidechain fits into the isoleucine binding pocket of the synthetase.[6,8,9] A limited number of semisynthetic analogs closely resembling PMA, with modifications at the left hand sidechain have previously been prepared and shown to be less biologically active than PMA.[15,16] Since the available data on inhibitors suggested that there was not an absolute requirement for an isoleucyl moiety to achieve tight binding in the enzyme active site, and the structural range of PMA analogs was quite limited, it appeared reasonable that further opportunities existed for discovering new inhibitors based on the PMA template.

Our approach was devised and carried out to rapidly synthesize and screen a large number of PMA analogs with divergent "left hand" sidechains not obtainable from known semisynthetic precursors. By exchanging the C9-C14 sidechain with an amine residue at C8 of the pyran nucleus of PMA, the truncated precursor **2**[18] was chosen as a template from which propagation of analog synthesis could be carried out. By treating **2** with pools of appropriate electrophilic reagents, followed by deprotection, it was possible to rapidly prepare mixtures of compounds for biological screening. Identification of active compounds was carried out via independent synthesis of the components of mixtures displaying activity below a preset threshold.



The synthesis of **2** (Scheme 1)[19] began with a modification of a 2 step sequence reported by Schonenberger.[20] The protocol involved conversion of the 3,4-acetonide of D-ribopyranose into a β-*C*-glycoside via a one pot Wittig–Michael sequence, followed by isomerization of the ketal protecting group to yield alcohol **3**. The secondary alcohol at C8 of compound **3** was then subjected to Mitsunobu inversion using azide as a nucleophile.[21] Subsequent treatment with lithium aluminum hydride effected reduction of both the ester and azido functions, and the free amine was protected as its *t*-butyl carbamate.



Scheme 1

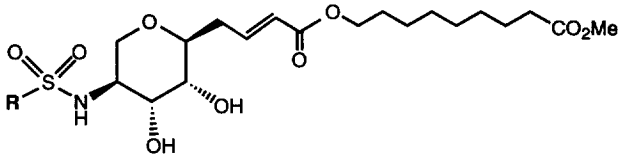
Reagents and conditions: (a) i. methyltriphenylphosphoranylidene acetate (1.5 equiv), THF, 0–23 °C, 18 h; ii. $NaOCH_3$ (cat), CH_3OH, 0 °C, 12 h, 60%; (b) 2,2-dimethoxypropane:THF (2:1), 55 °C, 12 h, separation of isomers, 75% (based on recovered starting material); (c) triphenylphosphine (1.2 equiv), diisopropylazodi-carboxylate (1.3 equiv), diphenylphosphoryl azide (1.5 equiv), THF, 0–23 °C, 18 h, 73%; (d) lithium aluminum hydride (8 equiv), THF, 0 °C, 20 min; (e) di-*t*-butyl-dicarbonate (4 equiv), NaOH (5 equiv), THF:CH_3OH:H_2O (2:1:1), 23 °C, 30 min, 75% over two steps; (f) TEMPO (cat), NaOCl (excess), KBr (0.5 equiv), CH_2Cl_2:H_2O (2:1), 0 °C, 2 h, 78%; (g) phosphonate (2 equiv), $LiN(TMS)_2$ (1.5 equiv), THF, 0–23 °C, 1 h, 40%; (h) TFA: CH_2Cl_2 (1:1), 23 °C, 30 min, 90%.

Stereoselective installation of the C2-C3 *E*-alkene was accomplished by primary alcohol oxidation followed by Horner–Emmons reaction with phosphonate **4**[22] to afford the carbamate protected precursor **5**.[23] Treatment of **5** with anhydrous trifluoroacetic acid gave the targeted amine **2**.[24]

Analog syntheses were carried out by treating aliquots of compound **2** with stoichiometric amounts of pools of 8–10 reagents under normal reaction conditions. The resulting materials were worked up in the usual manner, then deprotected with aqueous trifluoroacetic acid to yield the desired product mixtures.[25] The electrophilic reagents utilized in the above procedure included activated carboxylic acids, sulfonyl chlorides, isocyanates and aldehydes (reductive amination). These reagents were selected from commercially available materials to explore the size, shape, and polarity of sidechains introduced upon their reaction with compound **2**.

Biological screening[26] of the above compound mixtures identified an active pool of 8 sulfonamide derivatives. No significant activity was observed among analogs constructed with amine, carboxamide, or urea linkages. This indicates that basic substituents are undesirable and that a sulfonamide provides specific polar interactions while enforcing an appropriate sidechain geometry.

Table 1. Pseudomonic Acid Analogs.



Compound	R	IC_{50} (μM)[a]	MIC (μg/mL)[b]
PMA	-----	**0.0008**	**0.125**
6	**3-chloropropyl**	**0.1**	**50**
7	**3-chlorophenyl**	**0.3**	**25**
8	**methyl**	**>20**	**>100**
9	***n*-propyl**	**3.0**	**>100**
10	***n*-butyl**	**0.3**	**>100**

[a]vs isoleucyl tRNA synthetase
[b]*Staphylococcus aureus* (wild-type: ATCC 6538P)

The individual components of the active sulfonamide mixture were subsequently synthesized and screened. In this manner the 3-chloropropyl sulfonamide derivative **6** (Table 1) was identified as the active component. The related sulfonamide derivatives **7–10** were subsequently prepared and evaluated. Compound **7**, with the 3-chlorophenyl sulfonamide was found to have similar activity. The emerging SAR seems to indicate a preference for a lipophilic sidechain bearing an electronegative substituent as well as a steric requirement.

Although **6** is approximately 100 times less active than PMA, its discovery is nonetheless significant in light of the scarcity of reported modifications of the PMA left sidechain. The ability to replace the complex C9-C14 PMA left sidechain (4 stereogenic centers and a sensitive epoxide moiety) with a simple residue and retain weak activity represents the discovery of a novel compound series. Ongoing experimental and computational studies are directed towards an understanding of the observed activity as well as a further exploration of the SAR in this series.

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23. **5**: Clear oil; tlc $R_f = 0.31$ (silica gel, 3:2 hexane:ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (dt, J = 6.9, 15.5 Hz, 1H, H-3), 5.91 (d, J = 15.5 Hz, 1H, H-2), 5.03 (bd, $J \approx 9$ Hz, 1H, N-*H*), 4.19 (m, 1H, H-7), 4.11 (t, J = 6.6 Hz, 2H, H-9′), 4.02 (bd, $J \approx 8$ Hz, 1H, H-8), 3.71–3.80 (m, 3H, H-6, 9a, 9b), 3.67 (s, 3H, OCH_3), 3.31 (td, J = 3.1, 8.9 Hz, 1H, H-5), 2.60 and 2.30 (2 x m, 1H each, H-4a, 4b), 2.29 (t, J = 7.5 Hz, 2H, H-2′), 1.55–1.70 (m, 4H, H-3′, 8′), 1.49 (s, 3H, $C(CH_3)_2$), 1.46 (s, 9H, $C(CH_3)_3$), 1.36 (s, 3H, $C(CH_3)_2$), 1.30 (bs, 8H, H-4′, 5′, 6′, 7′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 166.4, 155.0, 144.6, 123.7, 109.8, 79.8, 77.6, 74.4, 73.8, 67.4, 51.4, 47.3, 35.5, 34.0, 29.1, 29.0, 28.6, 28.4, 26.4, 25.9, 24.9; HRMS (LSIMS) calcd for $C_{27}H_{45}NO_9Cs$ (M + Cs^+): 660.2149, found: 660.2132.
24. The amine **2** was generated and used immediately; however, no decomposition was observed after several weeks at room temperature in chloroform solution.
25. The compound mixtures were subjected to NMR, HPLC and mass spectroscopic analyses to determine their compositions. Most of the desired components were shown to be present in addition to smaller numbers of unidentified materials.
26. Samples were assayed for inhibition of *E. Coli* isoleucyl tRNA synthetase as described in ref 6. Bacterial growth inhibition assays were performed using standard broth dilution techniques.

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