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Synthetic studies towards the penicisulfuranols: Synthesis of an advanced spirocyclic diketopiperazine intermediate

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Abstract

The 2,5-diketopiperazine (DKP) moiety is a core feature of many natural products and medicinally relevant scaffolds. As part of our efforts directed towards a total synthesis of penicisulfuranol B, we have developed and report herein: (1) the preparation of an *N*-hydroxy diketopiperazine intermediate accessible via a molybdenum-mediated oxidation of a parent diketopiperazine, and (2) further synthetic studies leading to a novel spirocyclic dihydrobenzofuran-containing diketopiperazine.

Keywords

Penicisulfuranols; Diketopiperazine; Spirocycle; Hydroxamic acid; Oxidation

1. Introduction

The Penicisulfuranol family of natural products was isolated in late 2016 by Zhu and co-workers from the fungus *Penicillium jan-thinellum* HDN13–309; obtained from the root of *Sonneratia caseolaris* [1]. The disulfide-containing compounds of this family, have been found to display favorable cytotoxic properties towards HeLa and HL-60 cell lines exhibiting IC₅₀ values in the low μ M range. Penicisulfuranol B (**2**) is one of the more active members of the family displaying IC₅₀ values of 3.9 μ M and 1.6 μ M, respectively.

Although the biological activities of **1–6** are interesting, we were drawn to this family due to the formidable synthetic challenge that their unique molecular architectures present. A number of complex structural features define the penicisulfuranols: an oxadecalin system, an *N*-alkoxy diketopiperazine core, a spirocyclic dihydrobenzofuran moiety, and unusual disulfide bridge connectivity in **1–3**.

The penicisulfuranols are part of a much larger class of epipolythiodiketopiperazine (ETP) fungal toxins (e.g. **7–9**, Fig. 2) [2], which are defined by the central 2,5-diketopiperazine moiety and a bridging polysulfide. While the polysulfide bridge is usually located on the α -positions of the two amino acid residues that form the DKP (e.g. **8–9**, Fig. 2), ring expanded variants where the polysulfide bridge is located on the α -position of one residue and the β -

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Appendix A. Supplementary data

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position of the second residue (e.g. **1–3** and **7**, Figs. 1 and 2) are less common. Our current synthetic progress towards installing these polysulfide functionalities is presented herein, and highlights a unique, concomitant cyclization to furnish a spirocyclic dihydrobenzofuran moiety such as those found in compounds **1–7**.

In addition to this unique disulfide connectivity, the penicisulfuranols contain a central *N*-alkoxy DKP, a synthetically challenging functional group due to (1) the rather limited synthetic options for the preparation of *N*-alkoxy DKPs, and (2) the inability to further functionalize derivatives due to the instability of the nitrogen-oxygen (N–O) bond. Numerous examples of this latter reactivity can be found in the literature including work reported by Brown, Ottenheim, Baran, and Liu (Fig. 3) [3]. While several ETP natural products have been synthesized, the number of successful synthetic efforts towards ETPs containing the N–O moiety have been limited [4]. The most notable of these efforts is the total synthesis of the structurally-related natural product aspirochlorine (**7**) by Williams and co-workers, which to date, represents the only reported synthesis of a spirocyclic DKP natural product containing the N–O moiety [4a].

As one could anticipate from past precedents, in our investigations towards the penicisulfuranols, we encountered similar challenges while advancing intermediates containing the labile N–O bond. Given these difficulties, we opted to introduce the N–O bond via a molybdenum-mediated oxidation of a prefunctionalized DKP. The results of which are presented herein.

2. Results and discussion

As illustrated retrosynthetically in Scheme 1, our plan for preparing penicisulfuranol B (**2**) called for late-stage introduction of the oxazadecalin system and disulfide via oxidation of the pendant phenol and masked disulfide in advanced intermediate **20**. The latter was seen as arising from **21** via selective oxidation of the electron-rich aromatic ring and concomitant sulfur migration/spirocyclization; a process akin to the dehydrative sulfur migration employed by Danishefsky and coworkers in their synthesis of the aspirochlorine core [5]. Importantly, the plan calls for the introduction of the labile N–O bond on a DKP intermediate (**22**) prefunctionalized with all of the carbon atoms required for the synthesis.

In the forward sense we considered a number alternatives for the preparation of **22** and eventually employed an approach developed by Liu [6]. As illustrated in Scheme 2, this strategy calls for the use of bis-*N*-acyl DKP **24**, which can be implemented via a single-pot procedure involving an interrupted bis-aldol condensation [7,8]. Importantly, the use of **24** as the point of departure results in aldol products that undergo *N*-to-*O*-acyl migration/elimination reactions and deliver intermediate aldol-condensation products that have undergone deacylation of the proximal amide which, in turn, allows for regioselective functionalization. Applying this approach in our efforts toward the penicisulfuranols involved initial condensation of **23** and **24** to produce **25** which is poised for a selective methylation that delivers the second aldol substrate (**26**) [7,9]. Introduction of benzaldehyde **27** into the reaction mixture at this stage then delivers aldol condensation product **22** as the only isolable product in 36% overall yield for this multistep, one-pot sequence [7,10]. ¹H

NOESY analysis of the product indicated that the single stereoisomer that had been produced possesses the (*Z,Z*)-olefin geometry illustrated in **22**. Notably, in addition to containing the requisite carbon atoms, **22** possesses two exo-benzylidene moieties that are poised for conversion to thiols.

Having accessed **22**, we next attempted oxidation of the secondary amide to the corresponding hydroxamic acid. There is only sparse precedent within the literature for the direct conversion of a secondary amide in a DKP to an *N*-hydroxyl DKP [11,12]. Thus, we opted to adapt more well-described conditions developed by Sammes for the oxidation of secondary amides and lactams to hydroxamic acids via the intermediacy of the corresponding trimethylsilyl imidate [13]. As illustrated in Scheme 3, during our investigations with various reagents we were able to oxidize the parent DKP **22** to *N*-hydroxy DKP (**28**) with MoO₅(HMPA). In the initial screen, we explored oxidations with either stoichiometric or two-fold excesses of NaWO₄/UHP, NaWO₄/UHP/HMPA, MoO₅(HMPA) (Py), MoO₅(HMPA), MoO₅(DMF), and MoO₅(DMF)₂ [13,14]. As illustrated in Scheme 3, of these reagents, the most satisfactory results were obtained with MoO₅(HMPA). However, we were never able to achieve complete oxidation of the intermediate trimethylsilyl imidate of **22**, thus, upon work-up we obtained an inseparable mixture of **28** and starting material (**22**). To remedy this, treatment of the mixture with TBSOTf to afford the protected *N*-hydroxy DKP **29** was pursued, but also generated the TBS imidate of **22**. Selective deprotection of the unwanted TBS imidate of **22** in the presence of the desired product **29** was accomplished by stirring the mixture in a slurry of silica gel in DCM solvent. This ultimately allowed for the separation and isolation of **29** along with recovered starting material **22**. Overall, this process furnished protected *N*-hydroxy DKP **29** in 15% yield over the four steps from **22**, with a 13% recovery of **22**.

Having determined the feasibility of amide oxidation, albeit low yielding and unoptimized, we decided to shift our investigations toward installing the sulfur moieties. We were drawn to reports from Schmidt and Olsen describing the introduction of thioacetates onto bis-exomethylene-bis-*N*-methyl-containing DKP substrates using thioacetic acid as the sulfur source [15,16]. As illustrated in Scheme 4, we discovered that exposure of **22** to similar conditions results in the reasonably efficient and chemoselective incorporation of only a single thioacetate moiety. Thus indicating that more heavily substituted alkylidenes are tolerated, but the presence of a simple N–H bond is not (i.e., sulfur is only incorporated adjacent to the alkylated nitrogen). Further efforts to explore the chemistry needed to advance **22** revealed that deprotection can be accomplished under acidic conditions in the presence of an alkylation scavenger and that the derived phenol (**31**) can also serve as a substrate for thioacetate introduction, albeit with similar chemoselectivity.

To determine if indeed the lack of amide functionalization was thwarting introduction of the second thioacetate, **22** was permethylated by exposure to methyl iodide and Cs₂CO₃. The derived DKP (**33**) was deprotected, again using acid in the presence of a scavenger, to furnish bis-phenol **34** in 74% yield. Interestingly, exposure of **34** to the same thioacetic acid conditions that had been employed to advance either **22** or **31** resulted in the an unexpected chemoselective spirocyclization to afford two separable dihy-drobenzofuran diastereomers **35** and **36** in an approximate 1:2 ratio, respectively (Scheme 5) [17]. Of note, only the

unsubstituted phenol was observed to undergo the cyclization process, whereas, the bis-methoxylated phenol did not. The nucleophilicity of the bis-methoxylated phenol is likely attenuated due to an intramolecular hydrogen bond with the adjacent methoxy group on the aryl ring [18], thus, thioacetate incorporation proceeds preferentially over cyclization. Additionally, if cyclization precedes thioacetate incorporation, or a reversible process is at play, the stability of the formed spirocycle would dictate cyclization versus thioacetate incorporation. To probe potential mechanistic pathways and gain further insight into this process we attempted to induce cyclization in the absence of thioacetic acid employing only HCl in dioxane, but this resulted in decomposition of the starting material with no desired product being obtained. The relative stereochemistry of the dihydrobenzofuran spirocycle and thioacetate was determined by X-ray crystallography (Fig. 4).

3. Conclusions

In ongoing efforts toward a synthesis penicisulfuranol B we have developed an efficient one-pot method for generating a DKP intermediate containing the requisite carbon atoms and have demonstrated the viability of late-stage *N*-hydroxylation. Preliminary studies on the core structure have provided insight regarding eventual sulfur incorporation and revealed an interesting spirocyclization reaction that delivers a dihydrobenzofuran-containing DKP. Based on these results, future W6 efforts toward the penicisulfuranol family are being aimed at advancing thioacetate DKPs **30** and **32**. Results from these investigations will be reported in due course.

4. Experimental section

4.1. General procedures

Unless otherwise stated, all reactions were performed in flame-dried glassware under a nitrogen atmosphere, and reagents were used as received. The reactions were monitored by normal phase thin-layer chromatography (TLC) on Millipore glass-backed 60 Å plates (indicator F-254, 250 µM) using the indicated eluents. Reactions were also monitored by a Waters Acquity UPLC-MS equipped with a UPLC BEH C18 1.7 µm (2.1 × 50 mm) column, using H₂O:MeCN plus 0.5% formic acid with a 95/5 to 10/90 gradient over 9 min. Dichloromethane, dimethyl formamide, acetonitrile, and benzene were dried using a solvent purification system manufactured by SG Water U.S.A., LLC. Anhydrous HCl in dioxane was purchased from Oakwood Chemicals. Manual flash chromatography was performed using the indicated solvent systems with Silicycle SiliaFlash® P60 (230–400 mesh) silica gel as the stationary phase. Automated flash chromatography was performed on a Teledyne RF p UVeVis Ms Comp MPLC using the indicated solvent systems, and Teledyne RediSep® Rf normal phase disposable columns of the indicated size at the indicated flow rate. Normal phase preparative HPLC was performed with the indicated solvent system and 10 mL/min flow rate using a Sunfire Silica Prep 10 µm (10 250 µm) column to provide analytical samples. ¹H and ¹³C NMR spectra were recorded on either a Bruker Ascend™ 400 spectrometer autosampler or a Bruker Ascend™ 600 autosampler. Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent resonance and coupling constants (*J*) are reported in hertz (Hz). NMR peak pattern abbreviations are as follows: s =

singlet, d = doublet, dd = doublet of doublets, t=triplet, at = apparent triplet, q = quartet, ABq = AB quartet, m = multiplet. NMR spectra were calibrated relative to their respective residual NMR solvent peaks, CDCl_3 = 7.26 ppm (^1H NMR)/77.16 ppm (^{13}C NMR), DMSO = 2.50 ppm (^1H NMR). IR spectra were recorded on Bruker Platinum-ATR IR spectrometer using a diamond window. High resolution mass spectra (HRMS) were obtained in the Baylor University Mass Spectrometry Centre on a Thermo Scientific LTQ Orbitrap Discovery spectrometer using ESI or $-\text{ESI}$ and reported for the molecular ion ($[\text{M}+\text{H}]^+$ & $[\text{M}+\text{Na}]^+$ or $[\text{M}-\text{H}]^-$ respectively). Single crystal X-ray diffraction data were collected on a BrukerApex II-CCD detector using Mo-K α radiation (λ = 0.71073 Å). Crystals were selected under oil, mounted on micromounts then immediately placed in a cold stream of N_2 . Structures were solved and refined using SHELXTL.

4.2. Experimental procedures and data of synthetic intermediates

4.2.1. DKP 22—To a septum-capped, flame-dried flask containing a stir bar was added DKP 24 [7,8] (5.9 g, 29.7 mmol, 1.0 equiv), followed by DMF (28 mL), aldehyde 23 [7,9] (8.1 g, 29.7 mmol, 1 equiv), 6 g of 3 Å molecule sieves, and Cs_2CO_3 (29.0 g, 89.1 mmol, 3 equiv). The resultant mixture was stirred for 3 h at 25°C. The reaction was then cooled to -15°C with an ice/salt bath, and MeI (1.85 mL, 29.7 mmol, 1 equiv) was added and the reaction mixture was allowed to stir for 2 h. At this point, the reaction mixture was treated with the second aldehyde 27 [7,10] (6.3 g, 29.7 mmol, 1 equiv) and then heated to 95°C with stirring for 3 h. The reaction mixture was then allowed to cool to room temperature, quenched by the addition of 100 mL brine, and extracted two times with 500 mL EtOAc. The combined organic layers were washed three times with 1 L of brine and then dried with MgSO_4 , filtered and the solvent was removed to yield a crude product. The crude material was then purified by automated column chromatography: 220 g SiO_2 ; 30:70 to 50:50 (EtOAc:Hex) to provide 6.1 g (36% yield) of the title compound as a yellow foam: ^1H NMR (400 MHz, chloroform- d) δ 8.41 (s, 1H), 7.43 (d, J = 7.3 Hz, 2H), 7.39–7.29 (m, 8H), 7.27 (d, J = 1.5 Hz, 1H), 7.23 (dd, J = 7.3 Hz, 2H), 7.16 (s, 1H), 7.07–7.02 (m, 2H), 6.99 (s, 1H), 6.84 (d, J = 8.6 Hz, 1H), 6.68 (d, J = 8.7 Hz, 1H), 5.19 (s, 2H), 5.02 (s, 2H), 3.91 (s, 6H), 2.84 (s, 3H); ^{13}C NMR (101 MHz, chloroform- d) δ 159.5, 158.9, 155.7, 154.4, 150.9, 142.6, 137.1, 136.0, 131.1, 130.5, 130.3, 128.9, 128.7, 128.5, 128.4, 128.3, 127.9, 126.4, 125.3, 123.1, 121.9, 116.7, 114.0, 113.7, 107.3, 75.4, 71.5, 61.1, 56.2, 35.7; FTIR (thin film): 2934, 1680, 1620, 1593, 1491, 1449, 1423, 1373, 1341, 1278, 1092, 1046, 993, 750, 695, 472 cm^{-1} ; ESI-HRMS m/z . calc'd for $\text{C}_{35}\text{H}_{32}\text{N}_2\text{NaO}_6$ $[\text{M} + \text{Na}]^+$, 599.2158 found 599.2178.

4.2.2. DKP 29—To a flame-dried vial containing a stir bar was added DKP 22 (100.0 mg, 0.173 mmol, 1.0 equiv) followed by 0.5 mL of MeCN and BSA (51 μL , 0.2 mmol, 1.2 equiv). The vial was sealed and heated to reflux for 2 h. At this point, the solvent was removed in vacuo. The crude residue was dissolved in 2 mL of dry DCM and divided into 0.5-mL aliquots for the screening of oxidation conditions with $\text{MoO}_5(\text{L})_1(\text{L})_2$ complexes. In the absence of light, to one of the vials was added $\text{MoO}_5(\text{HMPA})_1$ (15.3 mg, 0.043 mmol, 2 equiv). The resultant mixture was stirred for 36 h at 25 °C while wrapped in foil in a dark fume hood. After this period of time, the reaction was quenched by addition of 2 mL of an aqueous 1 M Na_4EDTA solution. The mixture was further diluted with 6 mL of DCM and stirred for 4 h. The organic layer was then separated and the remaining aqueous portion was

extracted three times with 1.5 mL of DCM. The combined organic layers were dried with Na_2SO_4 and the solvent was removed in vacuo. Initial purification of the crude material by column chromatography with 40:60 EtOAc:Hex with 2.5% added AcOH provided a mixture of **28** and **22**. The mixture of **22** and **28** (23.6 mg, 0.04 mmol, 1 equiv) was transferred to a dry vial with 0.5 mL of dry DCM to which, TBSOTf (45.8 μL , 0.2 mmol, 5 equiv) and NEt_3 (32.8 μL , 0.24 mmol, 6 equiv) were added. This mixture was stirred at 25°C for 1 h then quenched by addition of 1.5 mL of water. The resulting mixture was extracted with 1.5 mL of DCM three times, the combined organic layers were dried and solvent removed under reduced pressure to afford a crude residue. This residue, which contained a mixture of an unwanted TBS-imidate and title compound, was subjected to a slurry of SiO_2 (500 mg) in 2 mL of DCM solvent for 14 h at 25 °C, then purified using a pipette column: 20:80 (EtOAc:Hex) to provide 3.6 mg (15% yield) of the title compound as a colorless film along with 3.2 mg (13% yield) of recovered starting material **22**. An analytical sample was obtained by prep HPLC using the following eluent; 20:80 (EtOAc:Hex): ^1H NMR (400 MHz, chloroform- d) δ 7.41 (d, J = 7.7 Hz, 2H), 7.37–7.29 (m, 6H), 7.24–7.18 (m, 4H), 7.16 (d, J = 5.2 Hz, 2H), 6.94 (d, J = 7.3 Hz, 1H), 6.89 (d, J = 9.0 Hz, 1H), 6.83 (d, J = 8.7 Hz, 1H), 6.67 (d, J = 8.6 Hz, 1H), 5.12 (s, 2H), 4.98 (s, 2H), 3.90 (s, 6H), 2.75 (s, 3H), 0.66 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (151 MHz, chloroform- d) δ 161.2, 157.8, 157.2, 154.5, 142.6, 137.1, 137.0, 132.6, 130.0, 129.4, 129.0, 128.9, 128.6, 128.4, 128.3, 127.8, 127.3, 125.4, 123.2, 121.7, 119.9, 118.1, 115.5, 111.4, 107.4, 75.3, 70.4, 61.1, 56.2, 34.7, 25.5, 18.1, 1.2, – 5.1; FTIR (thin film): 2928, 1687, 1624, 1595, 1493, 1449, 1425, 1328, 1281, 1255, 1206, 1139, 1094, 1063, 1011, 827, 811, 788, 750, 732, 696 cm^{-1} ; +ESI-HRMS m/z : calc'd for $[\text{M} + \text{Na}]^+ \text{C}_{41}\text{H}_{46}\text{N}_2\text{NaO}_7\text{Si}^+$ 729.2966, found $\text{C}_{41}\text{H}_{46}\text{N}_2\text{NaO}_7\text{Si}^+$ 729.2959.

4.2.3. DKP 30—To a septum-capped, flame-dried flask containing a stir bar was added DKP **22** (250 mg, 0.43 mmol, 1.0 equiv), followed by 4 M HCl in dioxane (3 mL), and AcSH (3 mL). The reaction mixture was then stirred at 25°C for 43 h, at which point, the volatiles were evaporated under reduced pressure. The crude mixture was dissolved in 50 mL of DCM and washed with 75 mL of sat. aqueous K_2CO_3 . The organic layer was then dried with Na_2SO_4 , filtered, and the solvent removed in vacuo to afford the crude material. Purification was achieved by automated flash chromatography: (12 g SiO_2 ; 20:80 to 50:50 (EtOAc:Hex); to provide 169.4 mg (60% yield) of the title compound as a yellow foam: ^1H NMR (400 MHz, chloroform- d) δ 8.48 (s, 1H), 7.40 (s, 1H), 7.37 (d, J = 7.2 Hz, 5H), 7.33 (d, J = 7.1 Hz, 3H), 7.29 (d, J = 7.1 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.09 (d, J = 7.3 Hz, 1H), 6.94 (t, J = 7.9 Hz, 2H), 6.87 (s, 1H), 6.73 (s, 1H), 6.42 (d, J = 8.7 Hz, 1H), 5.18 (d, J = 11.0 Hz, 1H), 5.10 (s, 2H), 4.82 (d, J = 11.1 Hz, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 3.22 (d, J = 13.8 Hz, 1H), 3.11 (d, J = 13.8 Hz, 1H), 2.96 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (101 MHz, chloroform- d) δ 193.3, 163.4, 158.8, 155.6, 153.6, 151.9, 142.0, 137.2, 136.1, 130.7, 129.8, 128.7, 128.7, 128.5, 128.3, 128.1, 127.6, 125.8, 125.3, 123.3, 121.7, 118.7, 114.2, 112.0, 107.4, 77.7, 75.8, 71.3, 61.0, 35.9, 32.1, 30.3; FTIR (thin film): 3271, 3031, 2937, 1690, 1632, 1597, 1494, 1451, 1368, 1282, 1259, 1166, 1099, 1038, 995, 950, 908, 881, 859, 803, 732, 697, 625, 570, 522, 456, 423 cm^{-1} ; +ESI-HRMS m/z : calc'd for $\text{C}_{37}\text{H}_{36}\text{N}_2\text{NaO}_7\text{S} [\text{M} + \text{Na}]^+$ 675.2141, found $\text{C}_{37}\text{H}_{36}\text{N}_2\text{NaO}_7\text{S} [\text{M} + \text{Na}]^+$ = 675.2141.

4.2.4. DKP 31—To a septum-capped, flame-dried flask containing a stir bar was added DKP 22 (1.0 g, 1.7 mmol, 1.0 equiv), TFA (18 mL), and pentamethylbenzene (565.6 mg, 3.8 mmol, 2.2 equiv). The reaction mixture was stirred at 25 °C for 20.5 h, at which point, the volatiles were removed under reduced pressure. The crude mixture was purified by automated flash chromatography: 40 g SiO₂; 30:70 to 80:20 (EtOAc:Hex); to provide 373 mg (31% yield) of the title compound as a yellow foam: ¹H NMR (600 MHz, chloroform-d) δ 7.34 (s, 1H), 7.28 (d, *J* = 8.3 Hz, 1H), 7.25–7.21 (m, 1H), 7.11 (d, *J* = 8.2 Hz, 1H), 7.03 (s, 1H), 6.95–6.91 (m, 1H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.52 (d, *J* = 8.8 Hz, 1H), 3.93 (d, *J* = 2.1 Hz, (s, 3H), 3.91 (3H), 3.07 (s, 3H). ¹³C NMR (151 MHz, chloroform-d) δ 153.3, 153.0, 148.1, 135.4, 134.1, 131.1, 130.3, 128.9, 128.6, 125.5, 124.4, 121.0, 120.7, 118.0, 117.2, 116.6, 114.3, 103.9, 61.2, 56.1, 35.9; FTIR (thin film): 3271, 1673, 1601, 1503, 1454, 1427, 1342, 1296, 1165, 1094, 1035, 971, 900, 794, 753, 695, 635, 523, 419 cm⁻¹; +ESI-HRMS *m/z*: calc'd for C₂₁H₁₉N₂O₆ 395.1249, found C₂₁H₁₉N₂O₆⁻ 395.1249.

4.2.5. DKP 32—To a septum-capped, flame-dried flask containing a stir bar was added DKP 31 (52.4 mg, 0.13 mmol, 1.0 equiv), followed by 4 M HCl in dioxane (1.2 mL), and AcSH (1.0 mL). The reaction mixture was stirred at 25°C for 24 h, at which point, the volatiles were removed under reduced pressure. The crude residue was dissolved in 50 mL of DCM and washed with 75 mL of sat. K₂CO₃. The organic layer was dried with Na₂SO₄, filtered, and the solvent was removed under reduced pressure to afford a crude residue, which was purified by automated flash chromatography: 4 g SiO₂; 40:60 to 80:20 (EtOAc:Hex); to provide 36.3 mg (57% yield) of the title compound as a yellow foam: ¹H NMR (400 MHz, chloroform-d) δ 9.92 (s, 1H), 8.50 (s, 1H), 7.21–7.14 (m, 1H), 7.10 (d, *J* = 7.2 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.88 (t, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 8.6 Hz, 2H), 6.30 (d, *J* = 8.7 Hz, 1H), 6.19–6.13 (m, 1H), 3.78 (s, 3H), 3.70 (s, 3H), 3.49 (d, *J* = 14.1 Hz, 1H), 3.35 (d, *J* = 14.1 Hz, 1H), 3.23 (s, 3H), 2.33 (s, 3H); ¹³C NMR (101 MHz, chloroform-d) δ 194.2, 164.3, 159.3, 152.9, 152.2, 148.8, 135.4, 133.0, 130.4, 126.4, 124.4, 121.0, 120.3, 116.9, 114.1, 111.2, 104.2, 77.8, 61.0, 55.8, 35.7, 32.2, 30.5; FTIR (thin film): 3232, 2934, 1765, 1689, 1613, 1506, 1466, 1377, 1279, 1200, 1037, 889, 758, 628 cm⁻¹; +ESI-HRMS *m/z*: calc'd for C₂₃H₂₄N₂NaO₇S [M+Na]⁺ = 495.1202, found C₂₃H₂₄N₂NaO₇S [M+Na]⁺ = 495.1221.

4.2.6. DKP 33—To a septum-capped, flame-dried flask containing a stir bar was added DKP 22 (5.9 g, 5.2 mmol, 1.0 equiv), followed by DMF (15 mL), and Cs₂CO₃ (1.86 g, 5.7 mmol, 1.1 equiv). The reaction mixture was cooled to -15°C with use of an ice/salt bath then MeI (355 μL, 5.7 mmol, 1.1 equiv) was added and the mixture was allowed to stir for 2 h. At this point, the reaction mixture was quenched by addition of 50 mL of brine and then extracted two times with 500 mL of EtOAc. The combined organic layers were washed three times with 500 mL of brine and then dried with Na₂SO₄, filtered, and the solvent removed in vacuo to afford a crude material that was purified by automated flash chromatography: 80 g SiO₂; 30:70 to 50:50 (EtOAc:Hex); to provide 2.1 g (80% yield) of the title compound as a yellow foam: ¹H NMR (600 MHz, chloroform-d) δ 7.40 (d, *J* = 7.3 Hz, 2H), 7.37 (d, *J* = 7.6 Hz, 2H), 7.32–7.26 (m, 3H), 7.25 (s, 1H), 7.22 (d, *J* = 4.9 Hz, 1H), 7.20–7.16 (m, 2H), 7.10 (s, 1H), 7.00–6.95 (m, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 6.68 (d, *J* = 8.7 Hz, 1H), 5.09 (s, 2H), 4.99 (s, 2H), 3.91 (s, 6H), 2.90 (s, 3H), 2.81 (s, 3H); ¹³C NMR (151 MHz, chloroform-d) δ

162.1, 162.0, 156.6, 154.4, 151.0, 142.6, 137.1, 136.5, 132.2, 131.0, 130.1, 128.7, 128.7, 128.5, 128.2, 128.1, 127.5, 125.4, 123.6, 121.5, 120.7, 117.4, 117.3, 112.2, 107.3, 75.4, 70.5, 61.2, 56.2, 34.7, 34.6; FTIR (thin film): 2941, 1681, 1621, 1593, 1492, 1448, 1423, 1373, 1338, 1280, 1244, 1197, 1171, 1093, 993, 802, 750, 696, 609, 464, 418 cm^{-1} ; +ESI-HRMS m/z : calc'd for $\text{C}_{36}\text{H}_{34}\text{N}_2\text{NaO}_6$ $[\text{M}+\text{Na}]^+ = 613.2315$, found $\text{C}_{36}\text{H}_{34}\text{N}_2\text{NaO}_6$ $[\text{M}+\text{Na}]^+ = 613.2315$.

4.2.7. DKP 34—To a septum-capped, flame-dried flask containing a stir bar was added DKP 33 (1.0 g, 1.69 mmol, 1.0 equiv), TFA (18 mL), and pentamethylbenzene (552.2 mg, 3.72 mmol, 2.2 equiv). The reaction mixture was stirred at 25 °C for 21 h, at which point, the volatiles were removed under reduced pressure. The crude residue was purified by automated flash chromatography: 24 g SiO_2 ; 50:50 to 80:20 (EtOAc:Hex) to provide 515.8 mg (74% yield) of the title compound as an orange foam: ^1H NMR (400 MHz, chloroform- d) δ 7.24–7.20 (m, 1H), 7.17 (d, $J = 7.5$ Hz, 1H), 6.92 (dd, $J = 12.0, 8.0$ Hz, 3H), 6.50 (d, $J = 8.8$ Hz, 1H), 6.25 (s, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.01 (s, 3H), 2.96 (s, 3H); ^{13}C NMR (101 MHz, chloroform- d) δ 162.4, 154.2, 153.0, 148.2, 135.4, 132.7, 130.7, 130.6, 130.5, 125.5, 120.9, 120.5, 117.7, 116.5, 116.1, 113.9, 103.9, 61.2, 56.1, 34.7; FTIR (thin film): 3297, 2980, 1676, 1614, 1504, 1461, 1429, 1351, 1300, 1163, 1098, 971, 756, 698 cm^{-1} ; -ESI-HRMS m/z : calc'd for $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_6$ $[\text{M}-\text{H}]^- = 409.1405$, found $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_6$ $[\text{M}-\text{H}]^- = 409.1384$.

4.2.8. DKPs 35 and 36—To a septum-capped, flame-dried flask containing a stir bar was added DKP 34 (41 mg, 0.1 mmol, 1.0 equiv), followed by 4 M HCl in dioxane (0.5 mL), and AcSH (0.5 mL). The reaction mixture was then stirred for 19 h at 25 °C, at which point, the volatiles were removed under reduced pressure. The crude mixture was dissolved in 25 mL of DCM and washed with 50 mL sat. aqueous K_2CO_3 . The organic layer was then dried with Na_2SO_4 , filtered, and the solvent was removed in vacuo to afford a crude material which was purified by automated flash chromatography: 12 g SiO_2 ; 0:100 to 80:20 (EtOAc:Hex); to provide 16.3 mg (34% yield) of compound 35. Major product and relative stereochemistry was unequivocally determined by X-ray crystallography: Analytically pure sample of compound 35 was obtained by prep HPLC using 50:50 EtOAc:Hex. ^1H NMR (400 MHz, chloroform- d) δ 7.15 (d, $J = 7.3$ Hz, 1H), 7.08 (t, $J = 7.7$ Hz, 1H), 6.86 (t, $J = 7.4$ Hz, 1H), 6.75 (d, $J = 8.7$ Hz, 1H), 6.54 (d, $J = 8.0$ Hz, 1H), 6.41 (d, $J = 8.7$ Hz, 1H), 6.04 (s, 1H), 4.10 (s, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.49 (d, $J = 13.9$ Hz, 1H), 3.42 (d, $J = 17.4$ Hz, 1H), 3.29 (d, $J = 13.9$ Hz, 1H), 3.15 (s, 3H), 2.72 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, chloroform- d) δ 194.7, 165.0, 164.1, 158.3, 152.2, 148.7, 128.2, 126.5, 125.2, 124.3, 121.3, 111.5, 108.5, 103.8, 94.7, 61.1, 55.9, 39.1, 36.2, 32.0, 30.6, 29.0; FTIR (thin film): 3368, 2960, 1673, 1613, 1505, 1466, 1429, 1387, 1280, 1239, 1099, 959, 869, 751, 630 cm^{-1} ; -ESI-HRMS m/z : calc'd for $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_7\text{S}^-$ $[\text{M}-\text{H}]^- = 485.1388$, found $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_7\text{S}^-$ $[\text{M}-\text{H}]^- = 485.1348$.

The reaction also produced 29.6 mg (61% yield) of diastereomer (**36**) as a yellow foam. An analytically pure sample of compound 36 was obtained using prep HPLC conditions using 50:50 EtOAc:Hex; conditions for single crystal growth: slow diffusion of Et_2O into DCM; ^1H NMR (400 MHz, chloroform- d) δ 7.12 (t, $J = 7.9$ Hz, 1H), 6.95 (d, $J = 7.3$ Hz, 1H), 6.92

(d, $J = 8.1$ Hz, 1H), 6.83 (t, $J = 7.4$ Hz, 1H), 6.75 (d, $J = 8.6$ Hz, 1H), 6.44 (d, $J = 8.8$ Hz, 1H), 6.04 (s, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.48 (d, $J = 13.6$ Hz, 1H), 3.19 (d, $J = 6.7$ Hz, 4H), 2.91 (d, $J = 16.9$ Hz, 1H), 2.75 (s, 4H), 2.35 (s, 4H), 2.23 (d, $J = 16.7$ Hz, 1H); ^{13}C NMR (101 MHz, chloroform- d) δ 193.9, 165.2, 163.8, 159.0, 152.4, 149.0, 128.7, 126.9, 124.1, 123.9, 121.3, 112.0, 109.7, 103.9, 94.0, 61.2, 56.3, 40.4, 35.9, 32.5, 30.5, 28.7; FTIR (thin film): 3368, 2980, 1672, 1610, 1505, 1464, 1427, 1387, 1325, 1279, 1240, 1098, 1070, 1039, 999, 969, 889, 869, 795, 734, 698, 628 cm^{-1} ; +ESI-HRMS m/z : calc'd for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{NaO}_7\text{S}$ $[\text{M}+\text{Na}]^+ = 509.1358$, found $\text{C}_{24}\text{H}_{26}\text{N}_2\text{NaO}_7\text{S}$ $[\text{M}+\text{Na}]^+ = 509.1358$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Penicisulfuranols A-F.

Fig. 2.
Representative ETPs.

Fig. 3.
Representative *N*-Alkoxy DKP Fragmentations.

Fig. 4.
X-ray crystal structure of **36**.

R = protecting group

Scheme 1.

Retrosynthetic analysis of penicisulfuranol B.

Scheme 2.
One-pot synthesis of bis-benzylidene DKP **22**.

Scheme 3.
Amide oxidation of bis-benzylidene DKP **22**.

Scheme 4.
Sulfur addition into bis-benzylidene DKP **22** and **31**.

Scheme 5.
Formation of an unexpected spirocycle.