Synthesis of Oxazole Analogs of Streptolidine Lactam

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The streptolidine lactam is an amino acid constituent of streptothricin antibiotics, which inhibit protein synthesis by targeting the bacterial ribosome but suffer from toxicity as a result of the basicity of the aminimidazole scaffold in the natural products. On the basis of the streptolidine structure, we designed and synthesized oxazole analogs with six- and seven-membered lactam rings as building blocks for RNA-targeted ligands. These analogs benefit from a dense network of hydrogen-bond donors and acceptors within a rigid nonplanar heterocyclic system that has attenuated basicity.

Introduction

Antibiotics that block bacterial protein synthesis by targeting ribosomal RNA (rRNA) share common traits despite the chemical diversity of these natural products.[1] Rigid arrangements of numerous hydrogen-bond donors and acceptors within a rigid molecular scaffold confer the affinity and selectivity of the RNA-binding ligands to the target.[2] Streptolidine lactam 1, which is a nonproteinogenic amino acid constituent of streptothricin antibiotics,[3] exemplifies this principle with an array of hydrogen-bonding interaction sites that line the edge of the rigid heterocycle (see Figure 1). Both trans- and cis-fused isomers have been found in natural products, however, the trans-fused system is more common.[4] The distribution of hydrogen-bond donor and acceptor sites at the edge of the heterocycle facilitates RNA targeting, but the high basicity of the aminimidazole moiety negatively affects its drug-like properties.

The aminimidazole moiety is an excellent anchor for the recognition of nucleobases in RNA folds. Evidence of this, for example, is the key role it plays in the binding of aminobenzimidazoles to an RNA target in the hepatitis C virus (HCV).[5] Indeed, streptothricin antibiotics have been shown to inhibit protein synthesis by direct interaction with the bacterial ribosome and blocking translocation of the peptidyl-tRNA complex.[6] Clinical use of potent streptothricin broad spectrum antibiotics is prevented because of its mammalian toxicity, as its use has been linked to indiscriminate protein binding and nephrotoxicity as a result of the high basicity of the compounds.[7] The electronic similarity of the aminobenzimidazole moiety to guanidine renders it quite basic with a pKₐ value of approximately 10. The crystal structure of an aminobenzimidazole ligand bound to an HCV RNA target reveals protonation of the imidazole moiety in the complex.[5b]

To exploit the dense network of hydrogen-bond donors and acceptors of 1 but remain within the limits of attenuated basicity, we designed oxazole analogs 2 and 3, which retain the hydrogen-bonding interaction sites of the streptolidine lactam but lack the guanidine-like basicity of the aminimidazole (see Figure 1). We recently developed the concise synthesis of oxazole analogs 2 for the preparation of synthetic RNA-binding ligands.[8] Herein, we describe the broadening of these efforts to access ring-expanded azepane oxazoles 3, which were designed to accentuate the nonplanarity of the RNA-targeting scaffold by increasing the lactam ring size. The avoidance of planarity prevents nonspecific intercalation and is considered beneficial for target selectivity in nucleic acid binding ligands.[2]

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Results and Discussion

For the synthesis of bicyclic oxazole lactams 2 and 3, we envisaged a nucleophilic ring opening of epoxy lactam precursors 4 and 5, respectively, with cyanamide followed by an intramolecular ring closure to give the oxazole analogs (see Scheme 1). Reports in the literature suggest that cyanamide readily converts noncyclic epoxides into oxazoles.\(^8\) The nucleophile attack occurs exclusively on the less-hindered side of a substituted epoxide substrate, which determines the regiochemistry of the formed heterocycle. We previously communicated the synthetic utility of this method to prepare oxazole analogs\(^1\).\(^9\) In the presence of a flanking, bulky tert-butyldimethylsilyloxy group, the addition of the cyanamide nucleophile to epoxy-δ-lactam 4 proceeded regioselectively, and ring closure of the oxazole by the alkoxide furnished the trans-fused product exclusively.

![Scheme 1. Retrosynthetic analysis of oxazole analogs (R = bulky protecting group).](Image)

To expand the scope of the oxazole analogs to develop RNA-targeting compounds, we explored the synthesis of azepane oxazoles such as 3 from the reaction of epoxy-ε-lactam 5 with cyanamide. We hypothesized that the seven-membered ring conferred sufficient rigidity to maintain the regioselectivity of the epoxide ring-opening and oxazole ring-closing sequence. For the synthesis of this precursor to azepane oxazole 3, we envisaged the formation of epoxy-ε-lactam 5 to occur from cyclohexenone 7 through a Beckmann rearrangement and epoxidation reaction (see Scheme 2). The preparation of precursor 7 commenced with the perchloric acid catalyzed hydrolysis of commercially available 1-methoxy-1,4-cyclohexadiene (8) to give 3-cyclohexenone 9\(^10\), which was oxidized with a peracetic acid to afford racemic epoxy ketone 10\(^*\) (see Scheme 3).\(^11\) The base-catalyzed ring-opening reaction of 10\(^*\) furnished the precursor cyclohexenone 7\(^*\) as a racemic mixture\(^11\).\(^11\) Although unsaturated lactam 6 was readily obtained through a Beckmann rearrangement of 7, the alkene position between the amide and alcohol functionality rendered 6 all but inert to an epoxidation reaction. Attempts using various peroxo acids, peroxides, and oxone failed to oxidize 6 and give 5.

![Scheme 2. Retrosynthesis of epoxy-ε-lactam precursor 5.](Image)

![Scheme 3. Preparation of precursor 7\(^*\) [Compounds 10\(^*\) and 7\(^*\) were racemates; mCPBA = meta-chloroperoxybenzoic acid, DCM = dichloromethane].](Image)

The inability to proceed with the reaction sequence through unsaturated lactam 6 led us to redesign the synthetic route to epoxy-ε-lactam 5 by carrying out the epoxidation step before the ring expansion (see Scheme 4). tert-Butylhydroperoxide (TBHP) in the presence of Triton B\(^12\) readily converted allylic alcohol 7\(^*\) into the syn-epoxide 11\(^*\), which was isolated as a racemate. The epoxidation of endocyclic allylic alcohols has been shown to proceed diastereoselectively to give the syn product, which is likely a result of the hydrogen bonding of the peroxide reagent to the same face as the hydroxy group.\(^13\) As alcohol 11\(^*\) was subsequently oxidized to meso-ketone 12, the stereochemical outcome of the epoxidation was of no consequence in the synthetic sequence to 5. The oxidation of 11\(^*\) by treatment with dichromate furnished meso-diketopiperazine 12, which was the precursor for the subsequent ring-expansion reaction. Guided by literature precedent for the conversion of an epoxy naphthoquinone into a benzazepine,\(^14\) we applied the Schmidt reaction to the ring expansion of 12 to give epoxy ketolactam 13\(^*\), and the stereochemistry of 13\(^*\) was confirmed by crystal structure analysis. The optimization of the conditions for the Schmidt reaction of 12 led us to introduce glacial acetic acid as a solvent, which allowed for better control of the heat generation and dissipation as well as prevented charring during the addition of the azide. Nevertheless, the yield of this step remained moderate. The borohydride reduction of ketone 13\(^*\) to give alcohol 5\(^*\) proceeded stereoselectively, with the adjacent epoxide directing the access of the hydride reagent to the less-hindered face to furnish the racemic anti-product 5\(^*\) exclusively. The stereochemistry of 5\(^*\) was confirmed by crystal structure analysis. The stereoselective reduction of α,β-epoxy ketones with sodium borohydride to give the anti product 5\(^*\) had been previously observed in the presence of calcium or lanthanum ions, which are thought to form a rigid bidentate com-
plex with the epoxy ketone and expose the less-hindered face to hydride attack.\[^{15}\] The inclusion of the epoxy ketone system to the rigid azepane ring of 13* might serve a similar function. The epoxide might force the azepane to adopt a concave/convex structure, in which the convex face is less hindered and thus serves as the preferred side for the hydride addition. Eventually, the reaction with tert-butyldimethylsilyl chloride (TBDMSCl) furnished silyl-protected epoxy-\(\varepsilon\)-lactam 14*, which was the precursor for the subsequent oxazole formation.

![Scheme 4. Preparation and silyl protection of epoxy-\(\varepsilon\)-lactam precursor 5* [Compounds 5*, 7*, 11*, 13*, and 14* were racemates; THF = tetrahydrofuran, PDC = pyridinium dichromate].](image)

We had previously prepared the silyl-protected oxazole analog (i.e., 16) of the streptolidine lactam through the reaction of epoxy-\(\delta\)-lactam 15 with cyanamide in dimethyl sulfoxide (see Scheme 5).\[^{8}\]

Analogous to the transformation of \(\delta\)-lactam substrate 15, the silyl-protected seven-membered ring precursor 14* was converted into protected azepane oxazole 17* by treatment with sodium cyanamide in dimethyl sulfoxide (DMSO) followed by an aqueous workup (see Scheme 6). The cyanamide nucleophile underwent a regioselective addition to the carbon that is adjacent to the amide carbonyl, as it had for six-membered ring substrate 15. Although the transformation proceeded slowly, side products from rearrangement were not observed. Unlike in the case of the six-membered ring analog, the main product of this reaction was desilylated azepane oxazole 3*, which was isolated in a fivefold excess amount over the protected derivative 17*. It was unclear why the desilylation occurred with the seven-membered analog but not for \(\delta\)-lactam 16.

The crystal structure analysis of the silyl-protected oxazole analog 16\[^{8}\] revealed packing contacts that engage pair-wise hydrogen bonds between neighboring heterocycles (see Figure 2), which is similar to the hydrogen-bonding interactions in nucleotide base pairs. All of the heteroatom groups at the edge of the oxazole lactam, which include the amide bond, are involved in such hydrogen bonds. This supports the original motivation for exploiting heterocycles such as 2 for targeting RNA folds with structures that contain a high density of hydrogen-bond donor and acceptor groups in a rigid cyclic system.

![Figure 2. Hydrogen-bonding pattern in the crystal structure of 16.](image)

The X-ray crystal structure of racemic 3*, which was crystallized as its hydrochloride salt, revealed the protonation of the oxazole nitrogen atom (see Figure 3). Although analog 16 was crystallized under neutral conditions, in which protonation was not observed, the pK\(_a\) value of the oxazole nitrogen is likely similar to that of the seven-membered ring analog 3. The crystal structure of the hydrochloride salt of 3* suggests that the hydrogen-bonding face of oxazole analogs such as 2 and 3 may maintain the arrangement of the hydrogen-bond donors and acceptors of

![Scheme 6. Preparation of the azepane oxazole analog (i.e., 3*) of streptolidine lactam (compounds 3*, 14*, and 17* were racemates).](image)
streptolidine lactam 1, yet within a less basic and, in the case of the seven-membered ring analog, a nonplanar heterocyclic system.

Figure 3. Crystal structure of 1* as a hydrochloride salt. A green sphere indicates a Cl− ion (see Supporting Information).

Conclusions

In summary, a synthetic strategy has been developed to prepare oxazole analogs of the natural streptolidine lactam scaffold of ribosome-targeting streptothricin antibiotics. Oxazole analogs retain the beneficial dense network of rigid hydrogen-bond donors and acceptors that mediate RNA recognition, yet within a heterocycle with attenuated basicity. The current study expanded previous work to include streptolidine oxazole analogs that contain a seven-membered ε-lactam system, which accentuates the nonplanarity of the RNA-targeting scaffold and reduces the potential of non-specific intercalation in nucleic acids. Seven-membered heterocycles are scarce among previously known RNA-binding ligands, despite the fact that they constitute attractive scaffolds for the assembly of RNA-directed functional groups within a rigid nonplanar framework of high atom economy. The synthetic procedure towards the azepane oxazole scaffolds described herein provides a starting point for the preparation of RNA-targeting compounds that contain a highly functionalized seven-membered heterocycle. Future studies will explore the synthesis of azepane oxazole derivatives for RNA targeting with modification at the hydroxy group and alkylation at the amino functionality.

Experimental Section

General Methods: Commercial reagents were used without any further purification. Acetone was distilled from potassium carbonate. Dry dimethylformamide was further purified by passing it through a silica gel column. Anhydrous dimethyl sulfoxide, methanol, and ethanol were purchased from AcroSeal. Nonaqueous reactions were carried out with dry, freshly distilled solvents and oven-dried glassware under anhydrous conditions and an inert atmosphere. Flash column chromatography was performed with silica gel 60 (230–400 mesh, Silicycle) with the indicated eluting solvent, yields refer to materials that were chromatographically and spectroscopically (1H and 13C NMR) homogeneous, unless otherwise stated. The NMR spectroscopic data were recorded with Varian Mercury 400 MHz, JEOL Unity 500 MHz, and Agilent V NMR S 500 MHz spectrometers that were outfitted with an XSegs cold probe. Low resolution mass spectra were obtained with a ThermoFinnigan LCQDECA-MS spectrometer. High resolution mass spectra were recorded with a VG 7070 HS or VG ZAB-ZSE mass spectrometer.

Cyclohex-3-ene (9)[10] Commercially available 1-methoxy-1,4-cyclohexadiene (8, 12.0 g, 0.109 mol) was added to a solution of carbon tetrachloride (30 mL) and water (75 mL). To this biphasic mixture, 70% perchloric acid (6 drops) was added with constant stirring by a magnetic stir bar. The reaction was kept at room temperature for 24 h. The mixture was then transferred to a separatory funnel, and the carbon tetrachloride layer was collected and dried with sodium sulfate. The solvent was removed under reduced pressure to give 9 as a colorless oil, which was used without further purification. 1H NMR (400 MHz, CDCl3): δ = 5.78 (m, 1 H), 5.64 (m, 1 H), 2.76 (m, 2 H), 2.38 (m, 4 H) ppm. 13C NMR (400 MHz, CDCl3): δ = 210.1, 127.1, 124.5, 40.0, 38.9, 25.8 ppm. HRMS: calcd. for C6H8ONa [M + Na]+ 119.0467; found 119.0468; Delta 0.9 ppm.

rac-7-Oxabicyclo[4.1.0]heptan-3-one (10)[11] A solution of cyclohex-3-ene (9, 10.5 g, 0.109 mol) in dichloromethane (250 mL) was placed under argon and cooled to 0 °C. Separately, a solution of 3-chloroperoxybenzoic acid (27.6 g, 0.120 mol) in dichloromethane (250 mL) was prepared. To the cyclohex-3-ene solution was added the 3-chloroperoxybenzoic acid solution (75% w/w in water) dropwise through a cannula over a period of 45 min and with constant stirring by a magnetic stir bar. After standing at 0 °C for 1 h, the ice bath was removed, and the reaction mixture was kept at room temperature for 24 h. The mixture was then transferred to a separatory funnel and washed with 5% Na2SO3 (250 mL), saturated NaHCO3 (250 mL), water (250 mL), and saturated NaCl (200 mL), respectively. The organic layer was collected and dried with sodium sulfate. The solvent was removed under reduced pressure to give racemic 10 as a pale yellow oil, which was used without further purification. HRMS: calcd. for C6H9O2 [M + H]+ 113.0597; found 113.0598; Delta 0.9 ppm.

rac-4-Hydroxy-cyclohex-2-ene (7*)[12] Racemic 7-oxabicyclo-[4.1.0]heptan-3-one (10*, 12.2 g, 0.109 mol) was dissolved in a 1:1 mixture of diethyl ether (65 mL) and dichloromethane (65 mL). Basic alumina (activity I, 38.9 g, 0.382 mol) was added with constant stirring by a magnetic stir bar. The reaction was kept under argon at room temperature for 2.5 h. Then, the mixture was filtered, and the filter cake was washed thoroughly with dichloromethane (50 mL). Removal of the solvent under reduced pressure furnished crude racemic 7* as a yellow oil. Silica gel column chromatography of the residue (hexanes/ethyl acetate, 1:1) yielded pure racemic 7* [6.8 g, 56% yield over 3 steps from 1-methoxy-1,4-cyclohexadiene (8)] as a colorless oil. 1H NMR (400 MHz, CDCl3): δ = 6.77 (d, J = 9.2 Hz, 1 H), 5.70 (dd, J = 9.2, 2.4 Hz, 1 H), 4.53 (dt, J = 2.4 Hz, 1 H), 4.35 (br. s, 1 H, OH), 2.31 (m, 1 H), 2.17 (m, 1 H), 2.09 (m, 1 H), 1.72 (m, 1 H) ppm. 13C NMR (400 MHz, CDCl3): δ = 200.1, 154.7, 128.7, 66.0, 35.5, 32.3 ppm. HRMS: calcd. for C6H9O2 [M + H]+ 113.0597; found 113.0598; Delta 0.9 ppm.
vacuo. Silica gel column chromatography (hexanes/ethyl acetate, 1:1) of the residue yielded racemic 11* (4.6 g, 83% yield) as a white crystalline solid. 1H NMR (400 MHz, CDCl3): δ = 4.22 (q, J = 4.4 Hz, 1 H), 3.62 (d, J = 0.1 Hz, 1 H, 3.48 (br. s, 1 H, OH), 3.26 (d, J = 4.4 Hz, J' = 2.4 Hz, 1 H), 2.54 (m, 1 H), 2.11 (m, 1 H), 1.94 (m, 1 H), 1.84 (m, 1 H) ppm. 13C NMR (400 MHz, CDCl3): δ = 204.6, 66.7, 58.8, 56.4, 34.5, 24.8 ppm.

7-Oxabicyclo[4.1.0]heptane-2,5-dione (12)\([\text{15}]\) To a solution of pyridinium dichromate (0.16 g, 0.57 mmol) in 80% aqueous ethanol (2.7 mL) over a period of 15 min with continuous stirring by a syringe, as the mixture was constantly stirred by a magnetic stir bar. The reaction solution was then placed under argon and cooled to 0 °C. Racemic 5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one (0.09 g) was dissolved in dichloromethane (10 mL), and the solution was added dropwise by a syringe into the reaction mixture. After standing at 0 °C for 1 h, the mixture was kept at room temperature for 12 h. The solution was then filtered through a short plug of silica gel to remove the excess amount of pyridinium dichromate. The plug was thoroughly washed with dichloromethane (200 mL), and the resulting solution was concentrated in vacuo. Silica gel column chromatography (250 mL) of the residue (hexanes/ethyl acetate, 2:1) yielded 12 (4.7 g, 86% yield) as a white solid. Data for 12* (0.20 g, 81% yield) as a crystalline, white solid. 1H NMR (400 MHz, CDCl3): δ = 7.38 (d, J = 4.0 Hz, 1 H), 4.01 (d, J = 8.8 Hz, J' = 6.0 Hz, 1 H, 3.37 (d, J = 8.8 Hz, J' = 4.0 Hz, 1 H), 3.17 (m, 2 H), 2.05 (m, 1 H), 1.67 (m, 1 H), 0.87 (s, 9 H), 0.09 (d, 6 H) ppm. 13C NMR (400 MHz, CDCl3): δ = 170.9, 70.7, 58.8, 35.6, 33.6, 25.9, 18.2, -0.4 ppm. HRMS: calcd. for C12H12NO3SiNa [M + Na]+ 280.1340; found 280.1339; Delta = 0.4 ppm.

rac-8-Oxa-3-azabicyclo[5.1.0]octane-2,6-dione (13)\([\text{14,17}]\) To a solution of 7-oxabicyclo[4.1.0]heptane-2,5-dione (12, 0.39 g, 3.08 mmol) in water (2.0 mL) and glacial acetic acid (4.05 mL) was added sodium azide (0.50 g, 7.71 mmol) with vigorously stirring, and then the reaction mixture was cooled to 5 °C. Ice-cold concentrated sulfuric acid (3 mL) was added at a rate of 4 drops per minute, and the mixture was kept at room temperature for 20 min. Ice water (4 mL) was added with constant stirring by a magnetic stir bar. Then, the mixture was transferred to a separatory funnel and extracted with dichloromethane (6 × 20 mL). The combined organic layers were dried with sodium sulfate and concentrated in vacuo. Silica gel column chromatography (hexanes/ethyl acetate, 1:2) of the orange oil yielded racemic 13* (0.27 g, 62% yield based on recovered starting material) as a crystalline, white solid.

CAUTION: This reaction, which generates toxic and carcinogenic substances, should be performed in a well-ventilated hood and behind a safety shield. Extreme caution during the course of the addition of sulfuric acid is necessary to obtain optimum yields and avoid charring the product. 1H NMR (400 MHz, CDCl3): δ = 7.23 (br. s, 1 H, NH), 4.45 (m, 1 H), 3.04 (m, 1 H), 2.77 (m, 2 H) ppm. 13C NMR (400 MHz, CDCl3): δ = 203.0, 168.9, 68.6, 55.8, 50.1, 34.8, 30.4 ppm. HRMS: calcd. for C12H12NO3SiNa [M + Na]+ 280.1340; found 280.1339; Delta = 0.4 ppm.

(3aR,8aS,8aR)-2-Amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (17*): A solution of sodium hydrogen carbonate (0.36 g, 5.6 mmol) in dimethyl sulfoxide (1.5 mL) was prepared and cooled to 10 °C. A solution of racemic 14* (0.13 g, 0.51 mmol) in dimethyl sulfoxide (1.95 mL) was then added by a syringe, as the mixture was constantly stirred by a magnetic stir bar. The reaction was kept at 10 °C for 1 h and then was warmed to room temperature, at which it was kept for an additional 23 h. After the reaction was concentrated in vacuo, water (4 mL) was added, and the resulting solution was titrated with HCl (0.1 M solution) at 0 °C to acidic. The mixture was then transferred to a separatory funnel and extracted with chloroform (8 × 3 mL) and both layers were collected. The water layer was concentrated, and the residue was recrystallized (methanol/water, 3:1) to give racemic 17* (0.08 g, 53% yield) as a white, crystalline solid. The combined chloroform layers were concentrated in vacuo. Silica gel column chromatography (ethyl acetate/methanol, 10:1) of the orange residue yielded racemic 17* (11% yield) as a white solid. Data for 17*: 1H NMR (500 MHz, D2O): δ = 5.06 (d, J = 12.0 Hz, 1 H), 4.62 (t, J = 12.0 Hz, 1 H), 4.14 (dt, J = 8.0 Hz, J' = 4.0 Hz, 1 H), 2.95 (2 mL), 1.56 (dt, J = 16.0 Hz, J' = 4.0 Hz, 1 H), 0.86 (m, 1 H) ppm. 13C NMR (500 MHz, D2O): δ = 168.3, 163.3, 60.8, 57.8, 36.7, 33.7 ppm. HRMS: calcd. for C12H12N2O3Na [M + Na]+ 280.0693; found 280.0695; Delta = 0.0 ppm. The constitution and stereochemistry of the compound were confirmed by crystal structure analysis (see Supporting Information).
FULL PAPER

K. D. Rynearson, S. Dutta, K. Tran, S. M. Dibrov, T. Hermann

CD$_3$(OD): $\delta = 4.59$ (d, $J = 11.6$ Hz, 1 H), 4.14 (dt, $J = 11.2$ Hz, $J' = 5.2$ Hz, 2 H), 3.92 (t, $J = 11.2$ Hz, 1 H), 3.36 (dt, $J = 9.2$ Hz, $J' = 2.8$ Hz, 1 H), 3.15 (dt, $J = 8.8$ Hz, $J' = 2.8$ Hz, 1 H), 2.04 (m, 1 H), 1.56 (m, 1 H), 0.94 (s, 9 H), 0.15 (d, 6 H) ppm. $^{13}$C NMR (400 MHz, D$_6$DMSO): $\delta = 172.8$, 161.7, 85.9, 74.3, 66.7, 37.3, 37.1, 26.5, 18.6, $-3.7$, $-4.1$ ppm. HRMS: calcd. for C$_{13}$H$_{25}$N$_3$O$_3$Si [M + H$^+$] 300.1738; found 300.1739; Delta 0.3 ppm.

Supporting Information (see footnote on the first page of this article): Spectra and crystal structures of compounds listed in the Experimental Section.

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