OL Organic Letters

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Total Synthesis of Ajudazol A by a Modular Oxazole Diversification Strategy

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Cite This: https://dx.doi.org/10.1021/acs.orglett.0c02188 Read Online

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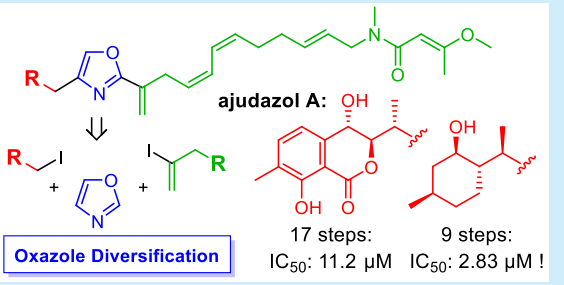
ABSTRACT: The total synthesis of the potent respiratory chain inhibitor ajudazol A was accomplished by a concise strategy in 17 steps (longest linear sequence). The modular approach was based on a direct oxazole functionalization strategy involving a halogen dance reaction for selective halogenation in combination with a challenging combination of sp^2–sp^2 and sp^2–sp^3 Negishi cross coupling reactions. The applicability of this strategy for analogue synthesis was demonstrated by the synthesis of a simplified as well as stabilized ajudazol analogue.



As exemplified by the ajudazols, potent respiratory chain inhibitors of myxobacterial origin, oxazoles are central structural features in a large number of natural products and bioactive agents.[1] Commonly, such types of heterocycles are prepared by a conventional cyclodehydration strategy, which involves condensation of an α-hydroxy amine with a carboxylate followed by oxidation of the resulting oxazoline. This biomimetic approach, as largely developed by the Wipf group,[2] guarantees mild reaction conditions and has been successfully applied in a wide variety of complex natural product total syntheses,[3] including ajudazol B.[4,5] However, the required linear sequence considerably increases the number of overall steps and complicates direct adaptability for useful analogue synthesis. As shown in Figure 1, the ajudazols are distinguished by a central oxazole core that is decorated by an unusual hydroxy-isochromanone moiety and a side chain with a (*Z*,*Z*)-diene and a terminal methoxybutenoic acid methylamide as characteristic features.[6] While ajudazol A (**1**) bears an exomethylene group next to the oxazole, ajudazol B (**2**) has a methyl group at this position. In combination with a very potent biological profile, characterized by selective inhibition of the mitochondrial NADH-dehydrogenase,[7] these intriguing molecular architectures have attracted a great deal of interest from the synthetic community,[5,8] culminating in one total synthesis of ajudazol B, which has been accomplished in 24 steps (longest linear sequence, 42 steps in total), previously described by our group.[5] In contrast, a direct oxazole functionalization would enable a much more convergent and modular approach and directly allows for application to analogue synthesis. Herein, we report the design, development, and application of a modular oxazole diversification strategy and the implementation of this approach for the total synthesis of ajudazol A (**1**) and the preparation of a simplified, stabilized, and much more readily available analogue.

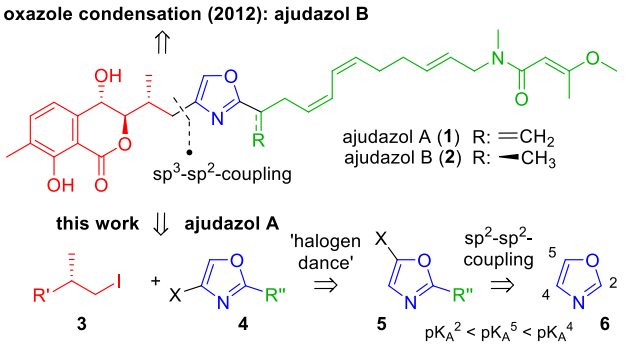


Figure 1. Retrosynthetic approach to the ajudazols by an oxazole diversification strategy and acidities of oxazole protons.

As shown in Figure 1, our retrosynthetic strategy was based on challenging sp^2–sp^2 and sp^2–sp^3 oxazole cross coupling reactions in combination with selective functionalizations of each oxazole position. This involved the effective use of the different heterocyclic pK_a values,[9] with H^2 being the most acidic and H^4 being the least acidic position, in combination with an adventurous halide rearrangement protocol. Consequently, lithiation of unsubstituted oxazole **6** selectively occurs at the C^2 site, giving intermediate **7** that was trapped with diphenyl disulfide toward thioether **8** [91% (Scheme 1)].[10] Subsequent deprotonation at the C^5 position and

Received: July 2, 2020

https://dx.doi.org/10.1021/acs.orglett.0c02188
Org. Lett. XXXX, XXX, XXX–XXX

Scheme 1. Selective Oxazole Functionalization

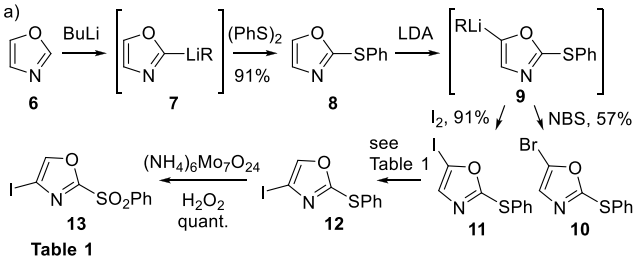
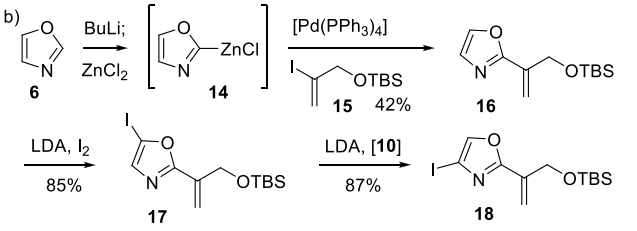


Table 1

entry	starting material	Catalyst (equiv)	equiv (LDA)	time	yield
1	**11**	-	1.60	50 min	**12** (25%), **7** (70%)
2	**11**	**10** (0.10)	1.80	63 min	**12** (88%), **7** (5%)



reaction of intermediate **9** with either NBS or iodine give C^5-halides **10** and **11**. Finally, to access the C^4 position selectively, a base-mediated halide displacement strategy was implemented. Such types of oxazole 1,2-halide rearrangements were first reported in 1953,[11] and these so-called halogen dance reactions involve treatment of 5-bromoxazoles with an excess of LDA, leading to migration of the bromine atom to the less accessible C^4 position, based on the higher acidity of the C^5 site.[12] While this procedure could be readily adopted to effectively move the bromine atom from position 5 to 4 (89%),[13] only low degrees of conversion were observed for the corresponding iodide **12** (Table 1, entry 1, in Scheme 1). As this more reactive halide was required for the ensuing cross coupling reactions (*vide infra*), a variant of the original protocol had to be devised. Presumably, the low yield arises from an undesired lithium−iodide exchange competing with the required C^4 deprotonation. Finally, this selectivity issue could be resolved by the addition of catalytic amounts of oxazole **10**, which suppresses undesired lithium−iodide exchange,[9] resulting in a clean rearrangement of iodide **11** toward 4-iodinated isomer **12** in high yields (88%, entry 2). Thioether oxidation to sulfone **13** was then realized in good yields with ammonium molybdate/H_2O_2. These sulfones may be directly replaced with organolithium compounds allowing for a facile introduction of Eastern subunits.[10] However, such reagents are not compatible with the authentic ajudazol isochromanone.[13]

Therefore, as an alternative a cross coupling strategy was pursued.[14]

As shown in Scheme 1b, for implementation of a cross coupling strategy lithiated oxazole **7** was transmetalated with $ZnCl_2$ and a Negishi cross coupling of resulting organyle **14** with vinyl iodide **15** was developed.[14] Subsequent C^5 iodination and halogen dance reaction of derived **16** toward 4-iodooxazole **18** proceeded smoothly following our previously developed protocols (74%, two steps), demonstrating the general usefulness of these procedures for selective oxazole derivatization. In general, alkyl cross coupling reactions continue to present formidable synthetic challenges.[15] In particular, only a slow process has been found in sp^3−sp^2 cross couplings with heteroaromatics, and no examples of oxazole alkyl cross couplings have been developed,[16,17] demonstrating the synthetic challenge of this key bond formation. Consequently, we first studied more readily available simplified surrogate **21**, having a similar substitution pattern, including the chiral β-methyl center of the original fragment. As shown in Scheme 2, it was obtained in three steps from commercial isopulegol **19** involving TBS protection, diastereoselective hydroboration/oxidation, and Appel reaction of derived **20**.

Scheme 2. sp^3−sp^2 Oxazole Cross Coupling Studies

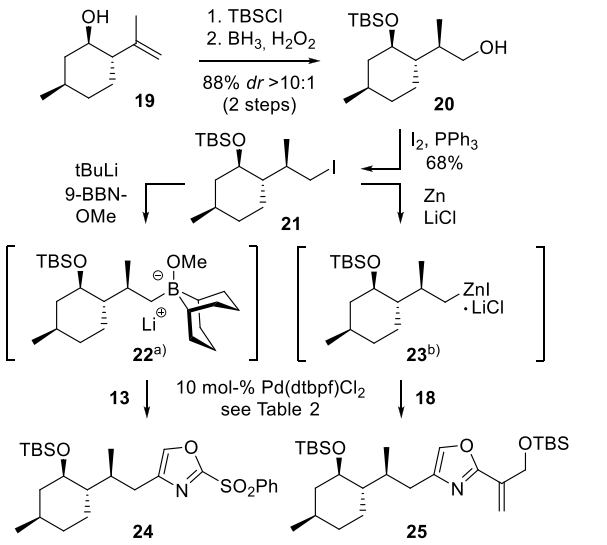


Table 2

entry	substrates	solvent	additives (equiv)	t (h)	yield (%)
1[c]	**22**, **13**	THF/toluene (1:1)	2M Na_2CO_3	20	16 (**24**)
2	**22**, **13**	THF/toluene (1:1)	2M Na_2CO_3	20	13 (**24**)
3	**22**, **13**	THF	2M Na_2CO_3	20	23 (**24**)
4	**22**, **13**	1,4-dioxane	2M Na_2CO_3	20	1 (**24**)
5	**22**, **13**	DMF	3M Cs_2CO_3	20	- (**24**)
6	**22**, **13**	DMF/THF (1:1)	3M Cs_2CO_3	20	7 (**24**)
7	**22**, **13**	THF	3M Cs_2CO_3	20	58 (**24**)
8	**22**, **13**	THF	3M Cs_2CO_3, $AsPh_3$ (0.3)	20	69 (**24**)
9[d]	**23**, **18**	THF	LiCl (1), $AsPh_3$ (0.30)	2	- (**25**)
10[d]	**23**, **18**	THF	LiCl (2), $AsPh_3$ (0.30)	2	- (**25**)
11	**23**, **18**	THF/DMI (2:1)	LiCl (2), $AsPh_3$ (0.30)	2	26 (**25**)
12	**23**, **18**	THF/NMP (2:1)	LiCl (2), $AsPh_3$ (0.30)	2	35 (**25**)
13	**23**, **18**	THF/NMP (2:1)	LiCl (2), $AsPh_3$ (0.30)	2	51 (**25**)
14	**23**, **18**	THF/NMP (2:1)	LiCl (3)	2	45 (**25**)
15	**23**, **18**	THF/NMP (2:1)	LiCl (3), $AsPh_3$ (0.30), NMI (1.30)	2	44 (**25**)

[a]Compound **22** was obtained by lithium−iodide exchange of the corresponding iodide **21** with *tert*-butyllithium (2.00 equiv) and 9-BBN-OMe. See the Supporting Information for details. [b]Compound **23** was obtained by insertion of Zn into the corrresponding iodide **21** with commercially available Zn powder in the presence of LiCl. See the Supporting Information for details. [c]The reaction mixture was heated to 70 °C for 18 h. [d]The reaction mixture was heated to 60 °C for 2 h.

Inspired by a few examples in complex natural product syntheses,[18] we first turned our attention to Suzuki reactions (Table 2, top part, in Scheme 2). As it soon became apparent that bromides were too unreactive, the coupling of iodide **13** with simplified Western subunit **21** was studied. To realize this coupling, Pd(dtbpf)Cl_2 proved to be a suitable catalyst for our requirements among those evaluated.[17] We assumed the reluctance of halooxazoles to oxidatively add to Pd(0) is one

https://dx.doi.org/10.1021/acs.orglett.0c02188
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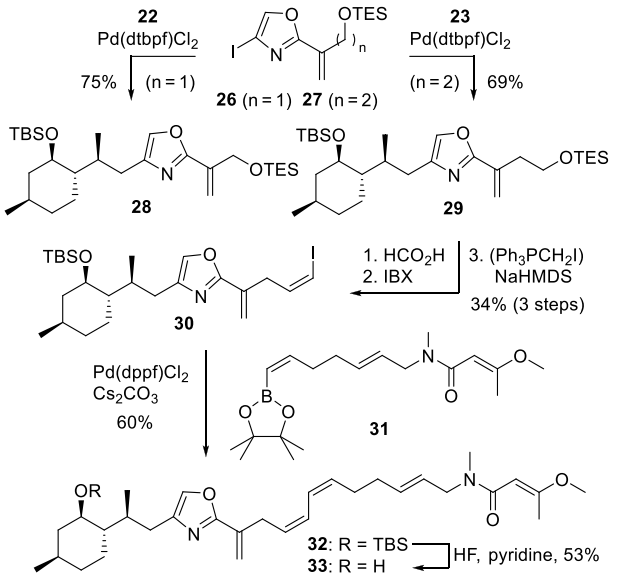
of the barriers to cross coupling the substrates and required heating to 70 °C (entry 1). Because β-hydride elimination occurs upon warming at 50 °C,[19] we investigated lower temperatures and discovered the ability of Pd(dtbpf)Cl_2 to forge oxazole **24** readily at room temperature (entry 2). The yield could be slightly increased in neat THF (entry 3), while lower degrees of efficiency were observed in more polar solvents like dioxane (entry 4) or DMF, giving mainly decomposition independent of the base (entries 5 and 6). Finally, useful yields were obtained with Pd(dtbpf)Cl_2 in the presence of Cs_2CO_3 in neat THF (58%, entry 7), which could be further increased by the addition of $AsPh_3$ (entry 8).[20]

As an alternative, an oxazole alkyl Negishi cross coupling was evaluated. Such an approach would involve a direct insertion of zinc into the organic halide, thus avoiding a lithiation process as required above.[21] Consequently, the coupling of elaborate oxazole **18** with iodide **21** was studied (entries 9−15). After initial unsuccessful attempts with conditions similar to those described above (entry 9) as well as related protocols with a large number of equivalents of LiCl to favor formation of heteroleptic zinc compounds (entry 10),[22] 1,3-dimethyl-2-imidazolidinone (DMI) as a highly polar co-solvent was added. We hoped to have a beneficial effect by enhancing the formation of higher-order zincates ($RZnX_3^{2-}$) as the putative active transmetalating agent.[23] This indeed proved to be effective, giving the desired alkyloxazole **25** with useful degrees of conversion (entry 11), which could be further improved by exchanging DMI with *N*-methylpyrrolidinone (NMP) (entry 12).[24] For further enhancement, we considered the possibility that each catalytic cycle would liberate one ZnX_2 molecule by transmetalation, which may then sequester halide ions from solution, due to their higher Lewis acidity,[25] and therefore, the Schlenk equilibrium would shift away from the formation of higher-order species required for coupling.[23] Indeed, the consequential increase in the number of LiCl equivalents had a beneficial effect (entry 13). In contrast, removal of $AsPh_3$ leads to incomplete conversion (entry 14), suggesting this co-catalyst is relevant for oxidative addition.[20] Also, *N*-methylimidazole (NMI), which had been described as an auxiliary reagent in previous alkyl−alkyl Negishi couplings,[24] had an inhibitory effect in our studies (entry 15), despite its structural similarities to DMI.

After having established these protocols, we turned our attention to application of these approaches for more elaborate target synthesis and first evaluated the preparation of simplified analogue **33** (Scheme 3). Notably, it contains the authentic butenamide motif from crocacin and the myxalamids,[26] which was considered to be part of the pharmacophore. Accordingly, oxazole **27** was elaborated toward **29**,[27] involving the two-step sequence established above, which proceeded uneventfully. Negishi cross coupling with surrogate **21** then proceeded in high yield (69%), supporting the usefulness of the protocol for complex alkyl cross coupling of oxazoles.[28] Completion of the analogue synthesis involved vinyl iodide **30**, which was obtained by selective removal of the primary TES ether under acidic conditions, IBX oxidation, and Stork−Zhao olefination of the intermediate aldehyde, which proceeded with full *Z* selectivity.[29] Finally, Suzuki coupling with known Eastern fragment **31**[30] using Pd(dppf)Cl_2 in the presence of Cs_2CO_3 and deprotection with buffered HF·pyridine provided synthetic analogue **33** in 32% yield over two steps.[31]

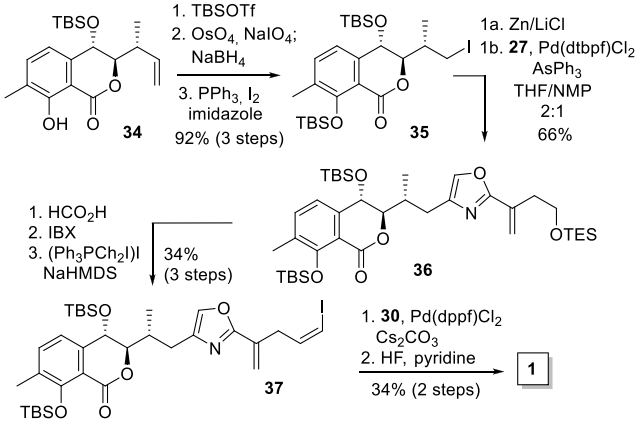
This approach could then be successfully adopted for the total synthesis of ajudazol A (**1**), as shown in Scheme 4.

Scheme 3. Implementation of Previous Protocols for Analogue Synthesis[a]



[a]Compounds **26** and **27** were obtained in a manner analogous to that of **16**. See the Supporting Information for details.

Scheme 4. Total Synthesis of Ajudazol A



Starting from isochromanone **34**,[30] which was obtained following tactics and protocols previously developed by our group,[5,13,32] the required Western building block **35** was obtained by TBS protection, oxidative alkene cleavage by an improved Lemieux−Johnson oxidation,[33] $NaBH_4$ reduction of the corresponding intermediate aldehyde, and Appel reaction of the derived alcohol in 92% yield over these three steps. Cross coupling with oxazole **27** likewise proceeded in high yield (66%), considering the functional complexity of the substrates and the great synthetic challenge posed by this fusion. Following our previously established protocols for our previous synthesis of analogue **33**, dialkylated oxazole **37** was then coupled with Eastern fragment **31**, to give synthetic ajudazol A (**1**). ^1H and ^{13}C NMR data were in agreement with the published data.[6]

Importantly, the first biological evaluation of ajudazol A (**1**) and simplified surrogate **33** revealed both compounds effectively inhibit T cell leukemia cells at micromolar concentrations (Jurkat cells),[34] confirming our hypothesis of

https://dx.doi.org/10.1021/acs.orglett.0c02188
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the terminal Eastern butenamide motif to be part of the pharmacophore. The simplified analogue **26** was even more potent (IC_{50} = 2.83 μM) than the parent natural product (IC_{50} = 11.2 μM).

More detailed biological studies will follow. In conclusion, this first total synthesis of ajudazol A highlights the potential of commercially available oxazole as a direct starting material for complex oxazole-containing natural products. It was accomplished in 17 steps (longest linear sequence), which compares favorably to a previous approach to this natural product class involving a conventional oxazole condensation strategy (24 steps). Highlights of this innovative strategy include specific oxazole functionalizations by the halogen dance reaction as well as sp^2–sp^2 and sp^2–-sp^3 oxazole cross couplings. The general usefulness of this approach for rapid and modular analogue synthesis was demonstrated by the facile preparation of a simplified and stabilized ajudazol analogue, which was obtained in only nine steps. It retains potent biological activities, suggesting the Eastern subunit is an essential part of the pharmacophore. This strategy may help to enable more detailed biological evaluations of the biological potential. Furthermore, the various strategies and tactics developed within this endeavor may be beneficial for the synthesis of various other complex oxazole architectures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02188.

Detailed experimental procedures, characterization, and copies of ^1H and ^{13}C NMR spectra of new compounds, including full details for all intermediates, synthesis of starting compounds (**26**, **27**, **31**, and **34**) as well as additional experimental information (see refs 13, 26, and 27) (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the DFG (ME-2756/9-1 and TRR 261) for generous funding and Andreas J. Schneider (University of Bonn) for excellent HPLC support.

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(27) Compounds **26** and **27** were obtained from **18**, or in analogy to **18**, demonstrating the generality of these protocols. See the Supporting Information.
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