Organic LETTERS

Letter

Cite This: Org. Lett. XXXX, XXX, XXX−XXX

pubs.acs.org/OrgLett

Synthesis of Grazoprevir, a Potent NS3/4a Protease Inhibitor for the Treatment of Hepatitis C Virus

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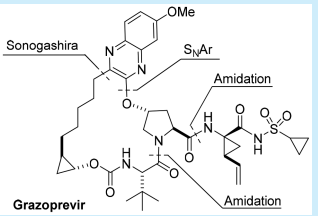
Supporting Information

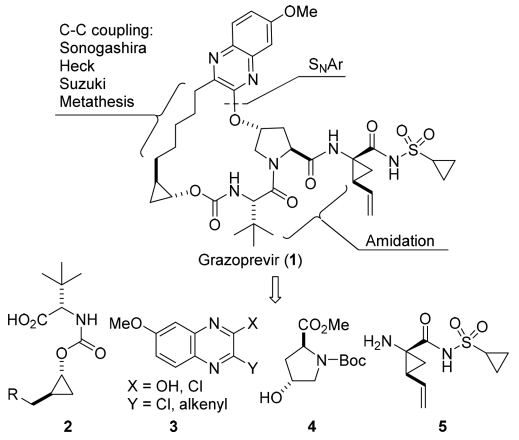
ABSTRACT: An efficient synthesis of grazoprevir is reported. Starting from four readily available building blocks, grazoprevir is prepared in 51% overall yield and >99.9% purity for pharmaceutical use.



Since the discovery of the hepatitis C virus (HCV) in 1989,[1] scientific advances in understanding of the HCV life cycle have led to the evolution of a series of direct-acting antiviral HCV therapies, resulting in significant improvement on virus cure rate and shortening treatment duration for diverse patient populations. Grazoprevir (**1**),[2] a potent NS3/4a protease inhibitor, in combination with the HCV NS5a inhibitor elbasvir, was recently approved as the novel therapeutic Zepatier for the treatment of HCV by the FDA and EMA.

Grazoprevir (**1**) features an 18-membered macrocycle and seven stereogenic centers. Retrosynthetic analysis of grazoprevir, employing multiple bond disconnection strategies, logically led to four building blocks, as shown in Scheme 1. Taking advantage of commercially available *N*-Boc-hydroxyproline ester **4**, it appeared synthetically attractive to establish the ether linkage between the hydroxyproline moiety and the grazoprevir skeleton via an S_NAr displacement of chloroquinoxaline **3** (X = Cl), while building blocks **2** and **5** could both be introduced by amidation reactions. To construct the backbone of the macrocyclic ring, we envisioned exploring several alternative protocols for C−C bond coupling with building block **2**, fitting our goal to develop a cost-effective and environmentally responsible process for the efficient preparation of grazoprevir. To this end, we recently reported a practical synthesis of the synthetically challenging *trans*-cyclopropoxy building block **2**, with a suitable functional group of choice in the cyclopropoxy ring,[3] which enabled us to explore various C−C coupling strategies including sp^2−sp^3, sp^2−sp^2, and sp^2−sp couplings and olefin metathesis, as outlined in Scheme 1. As such, a series of synthetic strategy permutations to form the macrocyclic ring, via C−C coupling or amidation, were accessible for investigation. This article describes the successful realization of the most practical strategy to assemble the described building blocks, with minimal functional group manipulation/transformation, resulting in an efficient and high-yielding synthesis of grazoprevir that achieves the high purity standards in the pharmaceutical industry for commercialization.

Scheme 1. Retrosynthetic Analysis of Grazoprevir



The strategy for closure of the grazoprevir macrocycle was ultimately responsible for the sequence of events and types of reactions employed to assemble building blocks **2**, **3**, and **4**. The chemistry applied to discover grazoprevir utilized a ring closing metathesis (RCM) reaction to form the macrocyclic skeleton (Scheme 2).[2b] The RCM process suffered a low yield (25%) due to the binding affinity of the catalyst with substrate **6** to form a stable chelated Ru complex.[4] Further investigation of the RCM strategy showed that this catalyst sequestering could be overcome using substrate **8** with an extended, pendant alkene chain off the quinoxaline moiety, affording a significant improvement of the RCM yield (71%).[4] However, on the basis of an overall cost consideration, the application of RCM to construct grazoprevir failed to provide the necessary benefits suitable for large scale preparation.

Received: October 4, 2018

DOI: 10.1021/acs.orglett.8b03173
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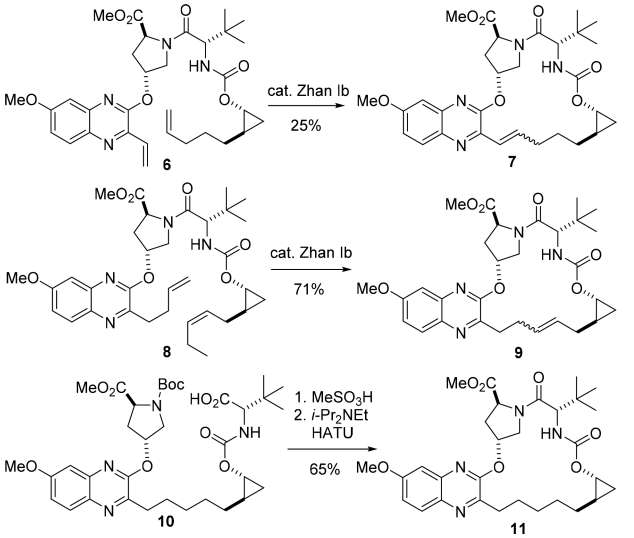
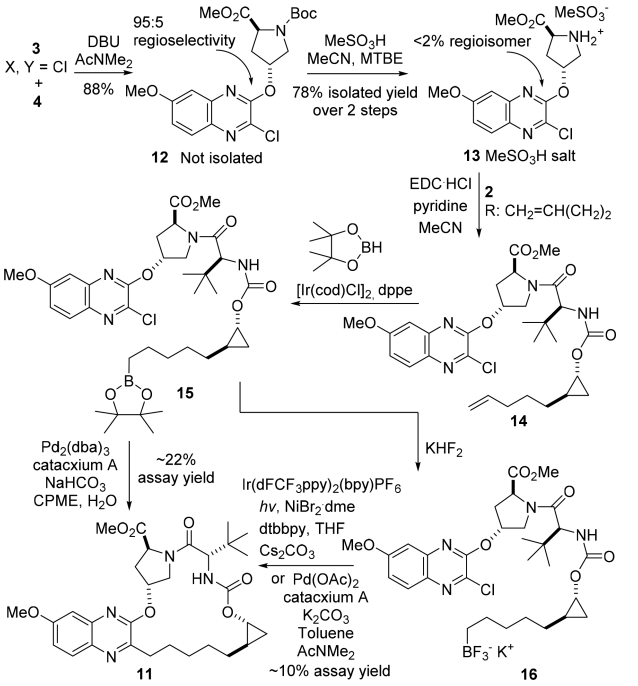
Scheme 2. Key Steps in the Early Syntheses of Grazoprevir



Scheme 3. Through-Process to Proline Chloroquinoxaline 13 and Macrocyclization via sp^2–sp^3 Coupling



The synthesis developed to support the early safety and clinical studies of grazoprevir relied upon a macrolactamization to form the macrocycle **11** (Scheme 2).[5] While the initial strategy for the preparation of precursor **10** through a Sonogashira coupling followed by hydrogenation proved effective, the liability of this synthesis was the incompatible reactivity of the electron-rich quinoxaline ring in **10** with the various conditions attempted for the requisite removal of the Boc group. Numerous impurities were formed through reaction of the quinoxaline moiety with cationic species generated under the deprotection conditions, adversely impacting the synthetic efficiency, yield, and overall process robustness. Given this observation, it was evident that removal of the Boc group, introduced from commercially available proline ester **3**, should be carried out earlier in the synthesis to suppress these side reactions, following formation of the S_NAr product **12** (Scheme 3), in which the quinoxaline moiety maintained its electron-deficient nature. In addition, we envisioned that access to the unprotected proline intermediate **13** from **12** would provide the opportunity to explore more promising synthetic permutations to prepare grazoprevir, as the building block carboxylic acid **2** could be incorporated in the core macrocyclic ring via amidation followed by a transition metal catalyzed C–C coupling with the chloroquinoxaline moiety or vice versa.

Interestingly, this early Boc deprotection approach created a new set of challenges. While the initial S_NAr reaction proceeded smoothly to form chloroquinoxaline **12**[5] in 88% yield and 95:5 regioselectivity, it was observed that the electron-deficient nature of the quinoxaline ring in the desired product **13** rendered it susceptible to competitive S_NAr reactivity at the remaining chloride position. When the Boc deprotection of **12** was carried out under acidic aqueous conditions or in alcohol solvents, S_NAr substitution of the chloride in the quinoxaline ring by the nucleophilic solvent (water or alcohols) to form the corresponding quinoxalinol or ether became significant.[6] Ultimately, employment of $MeSO_3H$ in the non-nucleophilic solvent, acetonitrile, proved crucial to developing a high-yielding Boc deprotection and afforded the salt **13** that crystallized directly from the reaction mixture as an acetonitrile solvate. With this result, a robust, two-step through-process of an S_NAr reaction followed by Boc deprotection was developed, from which the salt **13** was isolated in 78% yield[7] with good rejection of the regioisomer (98:2), while the chloro-substituted, electron-deficient quinoxaline ring remained intact.

With salt **13** in hand, we explored the feasibility of constructing the backbone of the 18-membered macrocycle via intramolecular sp^2–sp^3 coupling.[8,9] Selective hydroboration of amide **14** with pinacolborane in the presence of a catalytic amount of $[Ir(cod)Cl]_2$ /dppe afforded the boronate **15**, which was subjected to Suzuki–Miyaura coupling conditions to afford macrocycle **11** in 22% yield. Alternatively, treatment of boronate ester **15** with KHF_2[9a] yielded the corresponding potassium salt **16** that underwent cyclization via Molander's photoredox conditions in only 10% yield. Unfortunately, attempts to improve the cyclization yield by either pathway through extensive screening of Pd and Ni sources and ligands under numerous conditions proved unsuccessful.

Given the lack of progress with sp^2–sp^3 couplings, we turned our attention to applying an sp^2–sp coupling to install building block **2** (R = $HC{\equiv}C(CH_2)_2$) on quinoxaline **13** (Scheme 4).[10] With the nitrogen in the proline moiety unprotected, the thermostability of free base **13**, which was generated in situ under the basic conditions required for sp^2–sp coupling, proved concerning. In fact, attempts to apply the Sonogashira conditions[5] developed for the preparation of compound **10** resulted in complete decomposition of **13**. Further studies showed that use of an alcohol solvent was vital to develop a mild and efficient Sonogashira reaction addressing the thermoinstability of free base **13**, presumably the reduction capability[11] of alcohol solvent improved the catalyst stability and reactivity, which was evidenced by the significantly increased catalytic turnover number at mild temperature (Table 1, entry 2).[11] Carrying out the Sonogashira coupling in other solvents under various conditions resulted in an unsatisfactory yield and/or poor conversion. With methanol as solvent, we were able to decrease the reaction temperature to

DOI: 10.1021/acs.orglett.8b03173
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Scheme 4. Construction of Macrocyclic Core via Sonogashira Coupling−Macrolactamization

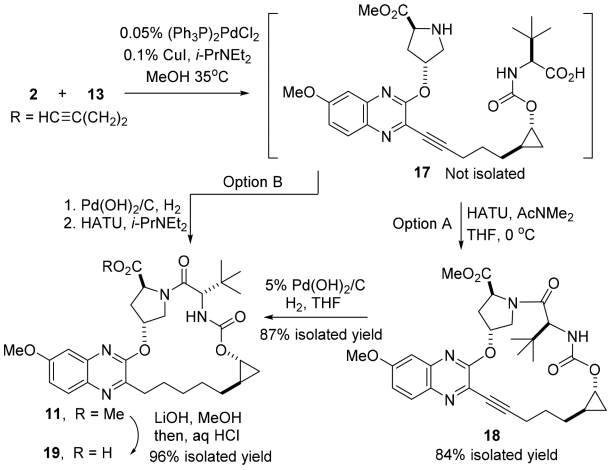


Table 1. Selected Results of Sonogashira Coupling of Chloroquinoxaline 13 with Alkyne Acid 2 (R = HC≡C(CH$_2$)$_2$)

entry	catalysts	solvents	cond	conv[a]	yield[a]
1	3 mol % $(Ph_3P)_2PdCl_2$, 6 mol % CuI	MeCN	50 °C, 5 h	>98%	85%
2	0.1 mol % $(Ph_3P)_2PdCl_2$, 0.5 mol % CuI	MeOH	35 °C, 5 h	>99%	98%
3	1 mol % XantPhos-Pd-G2, 30 mol % CuI	$AcNMe_2$	35 °C, 18 h	84%	78%
4	4 mol % $(Ph_3P)_2PdCl_2$, 30 mol % CuI	$AcNMe_2$	35 °C, 20 h	<20%	–

[a]Determined by HPLC analysis.[12]

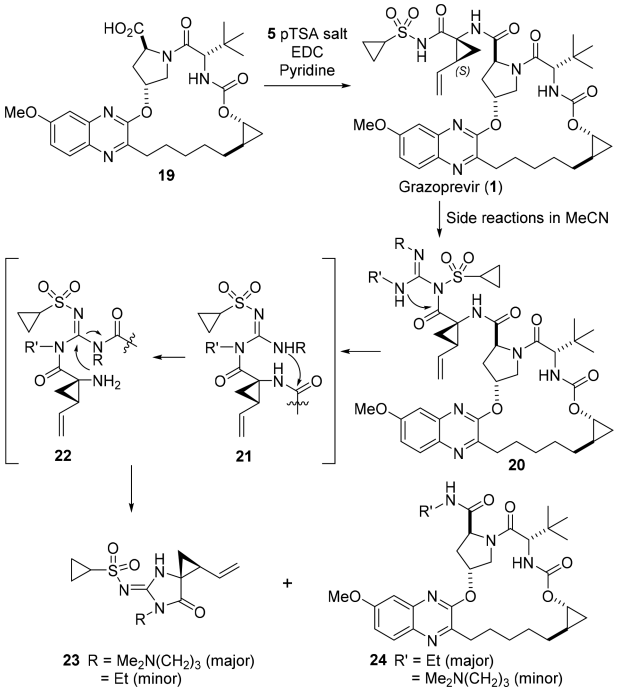
35 °C and the catalyst loading to as low as 0.05 mol % of air stable $(Ph_3P)_2PdCl_2$, one of the least expensive palladium catalysts, together with 0.1 mol % CuI. Under the optimized conditions, the Sonogashira coupling of **13** and **2** (R = HC≡C(CH$_2$)$_2$) proceeded smoothly in the presence of *i*-Pr$_2$NEt to yield the desired product **17** in 98% assay yield.

Without isolation of alkyne **17**, the Sonogashira reaction crude stream was solvent switched to THF/AcNMe$_2$ and treated with HATU to afford the desired macrolactam **18** in 84% isolated yield and >97% purity without any epimerization observed.[13] The corresponding regioisomer carried from **13** was rejected to <0.2%. Hydrogenation of alkyne **18** in the presence of 5% Pd(OH)$_2$/C in THF at 50 psi gave the desired **11** in >99% conversion. Upon removal of the Pd catalyst and concentration, macrocycle **11** was isolated from aqueous *i*-PrOH in 87% yield (Scheme 4, Option A). Alternatively, for Option B, which featured the reverse sequence of hydrogenation followed by macrolactamization, it was essential to treat the crude stream **17** with charcoal prior to hydrogenation to remove impurities poisoning the Pd catalyst in order to achieve the desired complete reduction (≥99%)[14] of alkyne **17** to the corresponding alkane. Although the subsequent macrolactamization proceeded well, Option A provided a more robust process and was selected as the macrocyclization process en route to grazoprevir.

After hydrolysis of ester **11** to the corresponding acid **19**, it was critical to identify the optimized conditions for the final amide coupling, while understanding degradation pathways that would both compromise the overall yield and form impurities that would require rejection in the final crystallization. Early efforts for the coupling of acid **19** with amine **5** were carried out in acetonitrile in the presence of EDC·HCl and pyridine.[5] However, this process was demonstrated to lack operational robustness and impurity control due to a series of significant competitive side reactions. In particular, to ensure isolating quality grazoprevir, a large excess of EDC·HCl (1.65 equiv) was required to achieve >99% conversion, because unconsumed **19** was poorly rejected under the isolation conditions. Furthermore, while the use of excess EDC·HCl resulted in high conversions, these conditions likewise led to the rapid formation of several byproducts that were also poorly rejected during the isolation. It was therefore crucial to quench the reaction as soon as the desired conversion had been achieved. Upon failing to do so, the quantities of the byproducts increased through continuous reaction of product **1** with EDC and resulted in concomitantly poor quality grazoprevir. This delicate balance between the side reactions vs rejection of impurities vs conversion rendered the control of product quality more challenging and necessitated additional studies to understand the pathways by which the multiple impurities were forming.

These studies exploring the competitive side reactions of EDC with grazoprevir led to identification of byproducts **20**, **23**, and **24**, the structures of which were unambiguously elucidated using NMR spectroscopy and LC/MS techniques. A plausible pathway for the formation of **23** and **24** via a novel rearrangement is depicted in Scheme 5. The EDC adduct

Scheme 5. Endgame and Plausible Pathways of Byproducts



guanidine **20** was shown to form in ∼30% yield when the reaction mixture was aged overnight. [1,3]-Carbonyl migration[15] would lead to the formation of intermediate **21**. Subsequent intramolecular nucleophilic attack would yield the electron-deficient guanidine **22** that could undergo fragmentation via a nucleophilic amine attack to afford **23** and **24**.[16]

DOI: 10.1021/acs.orglett.8b03173
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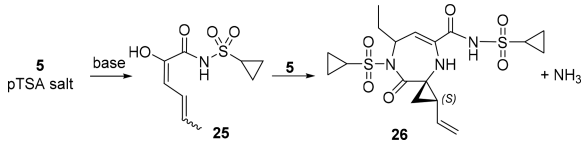
With this mechanistic insight in hand, a simple solution to suppress the formation of **20** and subsequent **23** and **24** was identified. By switching from the polar solvent acetonitrile to a much less polar solvent THF, which was believed to attenuate the nucleophilicity and/or acidity of the acyl sulfonamide moiety in **1**,[17] the nucleophilic addition to form adduct **20** was dramatically suppressed. Indeed, even aging the EDC reaction mixture in THF at ambient temperature for 1 week resulted in <2% of impurities **20**, **23**, and **24**.

Further optimization of the EDC coupling conditions was governed by the stability/reactivity of sulfonamide **5**. Unlike the stable pTSA salt **5**, the free base **5** proved to be unstable at ambient temperature,[18] with ∼40% observed decomposition overnight (Scheme 6).[12] This instability of the free base **5** was

Scheme 6. Decomposition of Sulfonamide 5



overcome by lowering the reaction temperature to 0−5 °C and by charging pyridine to a slurry of azeotropically dried acid **19** and **5** pTSA salt in THF. Subsequent treatment of the resulting homogeneous solution with EDC gave the desired **1** with an excellent purity profile, while the decomposition of **5** was suppressed to a minimal level (<1%). Of note is the extreme ease of operation of this endgame process at large scale without over-reaction/stability concerns. Upon aqueous citric acid workup, grazoprevir monohydrate was crystallized from aqueous acetone as its desired pharmaceutical form[19] in 94% yield and >99.8% purity with no impurities (>0.1%).[20]

In summary, an efficient synthesis of grazoprevir (**1**) has been described. The building blocks **2**−**5** are installed in an optimal and efficient sequence, eliminating the incompatible reactivity of the intermediates encountered in the alternative syntheses. The application of an effective Sonogashira coupling using the air-stable $(Ph_3P)_2PdCl_2$−CuI catalyst mixture served to construct the key macrocyclic backbone. The development of an optimal endgame process was accomplished with mechanistic understanding providing the requisite insight into helping suppress the competitive undesired reaction pathways. Starting from dichloroquinoxaline **3** (X, Y = Cl), with four isolated intermediates, grazoprevir was prepared in 51% overall yield and >99.8% purity for pharmaceutical use. By combining all the key elements required for a manufacturing process, this route has been successfully implemented for commercialization of grazoprevir.[20]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03173.

Experimental procedure, characterization data, and discussion (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the following colleagues of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, M. Maust, J. Hill, and M. Weisel of the High Pressure Laboratory and K. Belyk for experimental assistance, L. DiMichele, R. Reamer, and Dr. A. Buevich for assistance with NMR studies, Dr. J. Jo for HRMS analysis, and Dr. R. T. Ruck and D. Andrews for useful discussions.

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(6) Presumably, protonation of the chloroquinoxaline nitrogen increased its electron-deficient nature and sufficiently lowered the barrier for S_NAr reactivity. For the same reason, acidic aqueous workup of **13** proved to be undesirable.

(7) MeCN solvate **13** underwent desolvation upon drying.

(8) The use of intermolecular sp^2-sp^3 coupling is not attractive, due to the instability of the corresponding coupling product and free base **13** under these coupling conditions (*vide infra* in the text).

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(12) For details, see the Supporting Information.

(13) Intermolecular amidation was inevitable; however, it was significantly suppressed under the optimal lactamization conditions. The resulting oligomeric byproducts could be rejected to a nondetectable level in the subsequent isolated intermediates.

(14) Due to poor rejection of partially reduced alkene intermediate.

DOI: 10.1021/acs.orglett.8b03173
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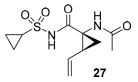

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(16) Formation of **23** was observed when amide **27** was treated with EDC.
(17) (a) Reichardt, C.; Welton, T. *Solvent Effects in Organic Chemistry*, 4th ed.; Wiley-VCH: Weinheim; 2011. (b) This solvent effect was also confirmed by varying the ratio of $AcNMe_2$ in THF.[12]
(18) Amine **5** has been used for amidation on large scale; however, studies on its stability/reactivity were lacking. Flick, A. C.; Ding, H. X.; Leverett, C. A.; Kyne, R. E., Jr.; Liu, K. K.-C.; Fink, S. J.; O'Donnell, C. *Bioorg. Med. Chem.* **2016**, *24*, 1937−1980.
(19) In the previous process,[5] it was necessary to recrystallize the initially isolated grazoprevir to obtain the desired pharmaceutical form.
(20) The entire process starting from **3** has been successfully carried out on large scale (>100 kg).

DOI: 10.1021/acs.orglett.8b03173
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