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Synthesis of Vaniprevir (MK-7009): Lactamization To Prepare a 22-Membered Macrocycle

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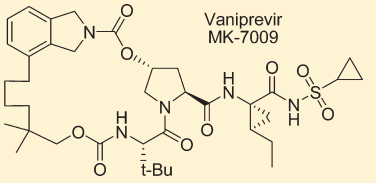
Supporting Information

ABSTRACT: Development of a practical synthesis of MK-7009, a 22-membered macrocycle, is described. A variety of ring-closing strategies were evaluated, including ring-closing metathesis, intermolecular palladium-catalyzed cross-couplings, and macrolactamization. Ring closure via macrolactamization was found to give the highest yields under relatively high reaction concentrations. Optimization of the ring formation step and the synthesis of key intermediates en route to MK-7009 are reported.



INTRODUCTION

Hepatitis C virus (HCV) affects an estimated 170 million people worldwide and is the leading cause for liver transplants.[1] Existing standard of care combines Pegylated interferon and ribavirin and only provides a modest cure rate.[2] This low cure rate is partially attributed to the fact that patient response is highly dependent on the genotype of the virus. To address this, various gene targets have been evaluated in order to identify new modes of treatment. Recent regulatory filings of boceprevir and telaprevir promise to improve the HCV therapy success rate. Despite these tremendous accomplishments, additional improvements in the area of genotype coverage, drug dose, and cure rate are still desired. One potential option is the HCV NS3/4a protease inhibitor vaniprevir (MK-7009), a compound in late phase clinical studies (Figure 1).[3−6] In order to support this promising candidate in preclinical and the initial clinical studies, a scalable process permitting access to MK-7009 was required.

The synthesis of MK-7009 employed by our colleagues in the Department of Medicinal Chemistry is illustrated in Scheme 1. MK-7009 is a 22-membered macrocycle constructed from four primary fragments: isoindoline (**1**), hydroxyproline (**2**), *tert*-butylglycine linker (**5**), and a cyclopropylsulfonamide (**8**). Following amide and carbamate bond formations with **1**, **2**, and **5**, the macrocycle is constructed via a high dilution ring-closing metathesis reaction catalyzed by ruthenium compounds.[7] Subsequent coupling of the cyclopropylsulfonamide (**8**) with macrocyclic acid yields MK-7009. After MK-7009 was identified as a clinical candidate, a closer evaluation of this synthesis was initiated with the goal of improving efficiency, yield, and productivity to support clinical needs. Herein, we describe the successful development of a practical synthesis of MK-7009 with particular emphasis placed on our efforts to develop an efficient ring-closing strategy.

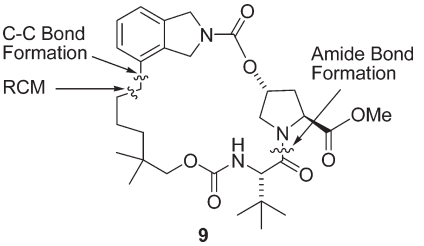


Figure 1. Macrocyclization strategies to prepare intermediate **9**.

RESULTS AND DISCUSSION

Employing the medicinal chemistry synthesis as a starting point for preparation of MK-7009, we began to evaluate potential methods for preparing macrocycle **9**. From the outset, it was clear that total route optimization could not be initiated until a cyclization strategy was identified, as this would define the overall synthetic approach. Consequently, we first detail our investigation into the identification of a suitable macrocyclization strategy, and second, we describe the synthesis and optimization of the premacrocyclization intermediates, macrocyclization reaction, and final steps to prepare MK-7009.

Ring-Closing Metathesis. While numerous methods for ring closure can be envisioned, we primarily focused on ring-closing olefin metathesis (RCM), intramolecular Pd-catalyzed cross coupling reactions, and macrolactamization (Figure 1). Initial efforts were placed on examination and optimization of the RCM route. Despite proceeding in good yields, the ring closure highlighted in Scheme 1 required high catalyst loadings and high dilution conditions (<3 mM). Initial efforts were placed on optimizing the reaction catalyzed by Neolyst M1 catalyst due to its easy access and relatively low cost. However, we observed that when catalysts loading was reduced or when the concentration

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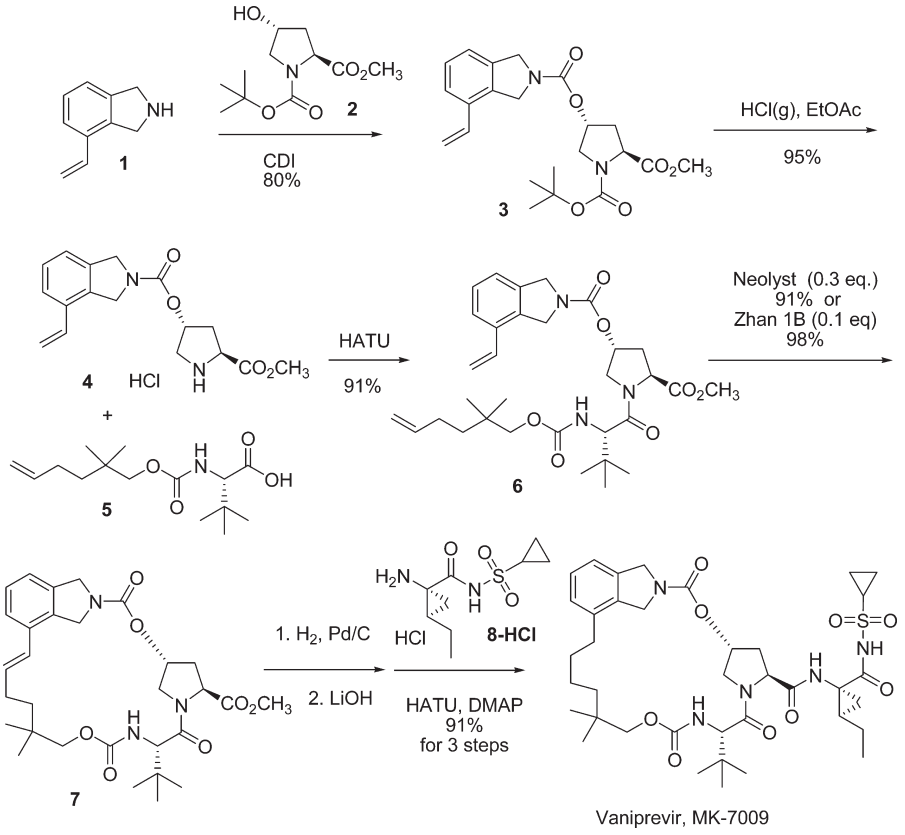
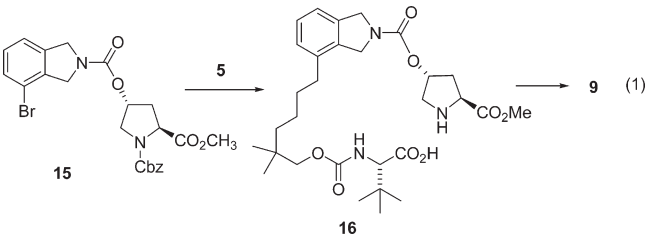
Scheme 1. Medicinal Chemistry Synthesis of Vaniprevir (MK-7009)



of the reaction was increased, substantially lower yields were obtained. Rigorous purification of the starting material was required to get complete conversion, as incomplete reactions could not be driven to completion by additional catalyst.[8] Catalyst removal after the reaction also posed challenges. In addition to their material high costs, many RCM catalysts are under patent protection. At this point, extensive work was needed to make the process amendable to scale up. Given these challenges, project timeline and material demands, further development of this specific strategy was halted and efforts were placed on identifying a more efficient ring-closing strategy.[9−12]

Palladium-Catalyzed Macrocyclization. Given the vast array of potential cross-coupling reactions and ligands available to effect these transformations, we opted to examine ring closure via palladium catalysis.[13−21] The intermediates used to prepare the RCM substrate were easily manipulated to generate molecules that could be subjected to palladium-catalyzed macrocyclization conditions. In particular, we have made intermediates **10**, **12**, and **13** in Scheme 2 and investigated Heck, Sonogashira, and Suzuki macrocyclizations.[22] For the Heck reaction, high-throughput catalyst, solvent, and base screen showed that most common conditions produced the desired macrocycle **11**. The best assay yield of the desired product **11** that was verified on 60 mg scale was 47%, which was achieved under high dilution conditions. Competing oligomerization, dehydrobromination, and 21-membered ring formation were encountered. For the Suzuki cyclization, we investigated very briefly the 9-BBN adduct **13** under a few common reaction conditions. Low reactivity and side reactions from the substrate **13** led to only a trace amount of product as detected by LC−MS. For the Sonogashira cyclization, even under high dilution conditions, a substantial amount of dimer was observed by LC−MS and the assay yield of the desired product **14** was only 34%. While continued optimization would likely lead to improved yields, ring closure via macrolactamization, a route being pursued in parallel, began to yield better results.

Macrolactamization. Cyclization via lactam formation is a frequently employed strategy in ring constructions.[23−28] As such, we investigated closing the macrocycle at proline and *tert*-butylglycine position (eq 1). Prior to investing efforts to optimize the synthesis of the precursors for macrolactamization, intermediate **16** was prepared on a small scale using a modification of the medicinal chemistry protocol. We were pleased to find that use of EDC with HOBt in DMF facilitated conversion to **9** in purity and yield superior to those obtained with the RCM and Pd-catalyzed cyclizations. Importantly, this scouting reaction served as proof of the effectiveness of the macrolactamization approach. Subsequent emphasis was therefore placed both on optimizing the macrocyclization reaction and on preparation of the macrolactamization precursors **5**, **15**, and **16** (eq 1).



(1)

Synthesis of the Heck Coupling Partners 5 and 15. As shown in eq 1, coupling of intermediates **5** and **15** was viewed as

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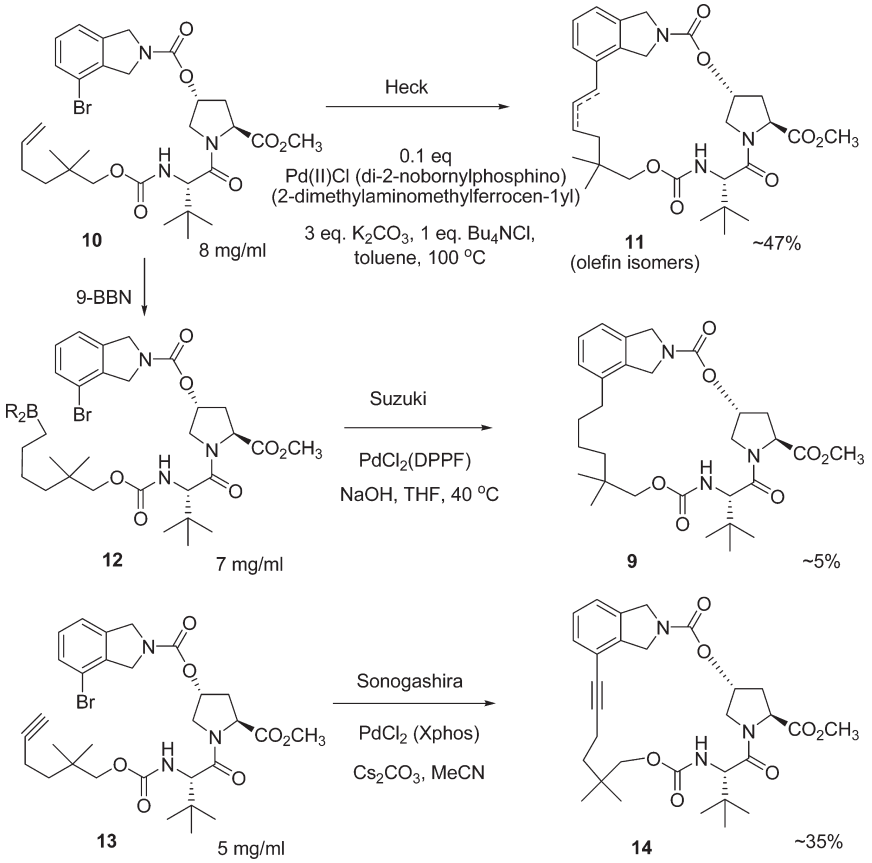
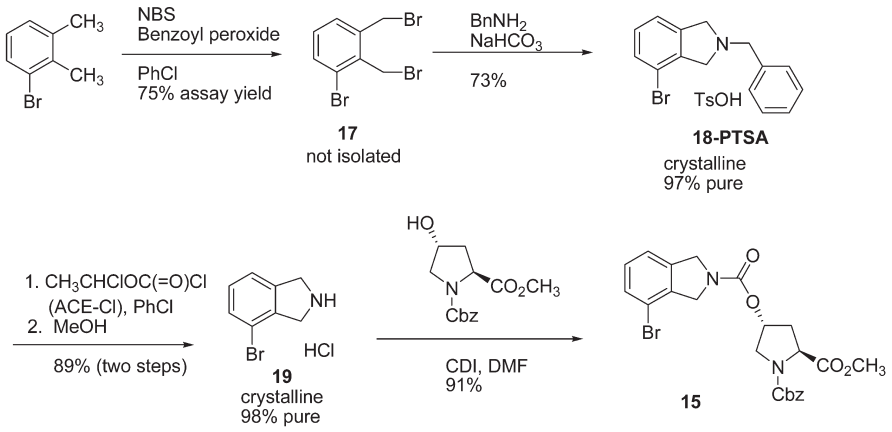
Scheme 2. Macrocycle Formation via Heck, Suzuki, and Sonogashira Reactions



Scheme 3. Optimized Process for Synthesis of Intermediate 15



the route to premacrocycle **16**. While alternate methods for connecting these two fragments are possible (vide infra), we initially focused on generating this bond through a Heck reaction and subsequent hydrogenation. The chemistry to prepare intermediates **5** and **15** involved modification and optimization of the medicinal chemistry precursors, as shown in Schemes 3 and 4. Notable changes in the synthesis of *N*-benzylisoindolene **18** include replacement of carbon tetrachloride with chlorobenzene in the bromination reaction and crystallization of the tosylate salt directly from the cyclization reaction in contrast to distillation of the corresponding free base. Subsequent debenzylation followed by CDI-mediated carbamate formation with Cbz-protected hydroxy proline ester gives **15**, which was isolated directly from DMF/water by crystallization. The Cbz protecting group was used in place of the Boc group to streamline the synthesis as hydrogenation of the macrolactamization precursor would both reduce the Heck alkene product and cleave the Cbz group.

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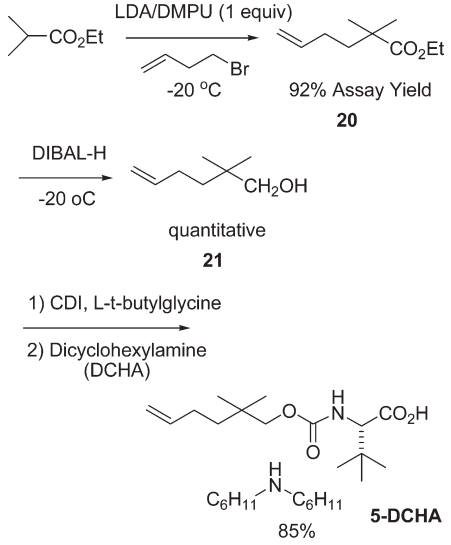
The *tert*-butylglycine derived linker **5** was prepared in three steps with one isolation (Scheme 4). Low-temperature deprotonation and alkylation of ethyl isobutyrate produced the desired ester **20** in good assay yield. Following aqueous workup, this crude solution was taken into a DIBAL-H reduction to generate alcohol **21**, which was then treated with CDI and *tert*-butylglycine to produce **5**, which was isolated as the dicyclohexylamine salt (**5-DCHA**).

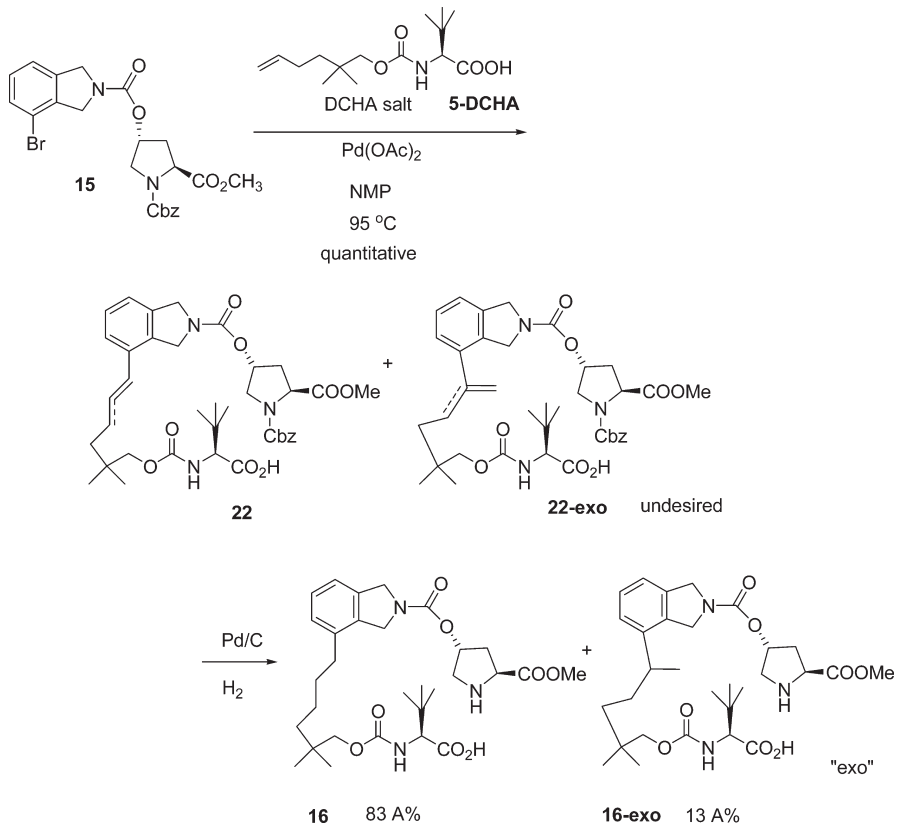
Heck Reaction and Hydrogenation. We were pleased to find that a variety of ligands in combination with Pd(II) and Pd(0) sources catalyzed formation of **22** (Scheme 5). In general, these reactions proceeded in greater than >85% assay yield of the desired linear olefin product and typically 10−12% of the undesired nonlinear olefin regioisomers (**22-exo**) regardless of the ligand identity.[29] Despite an exhaustive ligand and solvent screen, the ratio of regioisomers could not be changed, suggesting that the reactive catalyst either does not contain a ligand or that the regiochemistry-determining step is not influenced by ligand.[30] Intrigued by the observation that nearly every ligand gave the same yield and regioisomer ratio, $Pd(OAc)_2$ without added phosphine ligand was examined for the cross-coupling reaction. In this case, similar yields and regioisomer ratios were obtained *without* ligand. This suggests that productive bond formation is likely going through ligandless palladium, thereby explaining the results from our initial screen where ligand was found to have minimal impact on yield or selectivity. Following additional screening, we determined that $Pd(OAc)_2$ loaded as low as 0.75 mol % in NMP catalyzed formation of **22** in identical yield and regioisomer ratio to the reactions optimized with ligand.[31−33]

The desired Heck product was not isolated from the reaction mixture but taken directly into the subsequent hydrogenation (Scheme 5). In most cases, the residual palladium from the Heck reaction was sufficient to catalyze hydrogenation of Heck reaction products; however, conversions typically stalled at ∼95% by HPLC (area %). It was deemed critical to take the hydrogenation to >99% conversion to control process impurities downstream (vide infra). Therefore, in order to ensure acceptable conversion (>99%), a 5% by weight charge of 10% Pd/C was added prior to introduction of hydrogen. With this added catalyst charge, the hydrogenation was typically complete (≥99.8% conversion) in <12 h. Attempts to crystallize the premacrocyclization

Scheme 4. Optimized Synthesis of Olefin Linker 5



Scheme 5. Preparation of Macrolactamization Precursors via Heck and Hydrogenation Reactions



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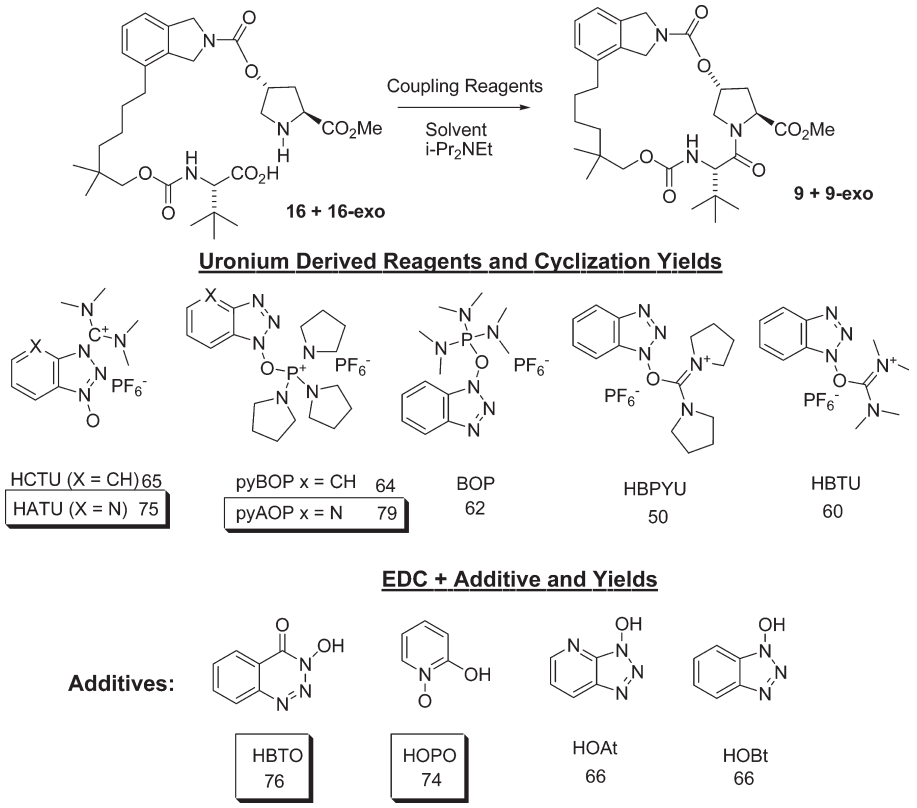

Figure 2. Selected list of coupling agents examined in the macrolactamization reaction.

intermediate **16** were unsuccessful. We thus opted to take the crude reaction mixture directly into the macrocyclization, which would require removal of Heck, hydrogenation, and macrocyclization impurities after the cyclization reaction.

Macrolactamization Optimization. Initial priority was placed on identifying an optimum amide bond forming reagent. This screening was done under high dilution conditions to minimize formation of oligomeric products. In particular, the hydrogenation reaction mixture which was in NMP solvent was diluted with different solvents to obtain a final concentration of 10 g/L (**16**/solvent).[8] A selection of the amide bond forming reagents examined is shown in Figure 2 and were either uronium-derived reagents or a mixture of EDC with an in situ activating agent.[34] Complete conversion of starting material was observed in nearly all cases; however, the corresponding assay yield and reaction time varied dramatically with the identity of the coupling reagent. In general, the uronium-derived reagents gave higher yields and shorter reaction times compared to the EDC derived reagents. For example, HATU gave the highest assay yield within a very short time frame (100% conversion, 75% assay yield, < 5 min).[35] The EDC-mediated cyclization, in particular with HOPO,[36] also produced **9** in good yield; however, reaction times were significantly longer (~12 h). Note that the assay yield reported is of the desired macrocycle and does not include the contribution from regioisomer cyclization (~10% assay yield).[37] The remaining 10% is a mixture of dimeric, trimeric, and oligomeric macrocycles, which were identified by mass spectrometry. Reaction temperature and solvents were also examined. In general, strongly polar aprotic solvents, like DMF, gave the best yields, while acetonitrile and THF could also be used with approximately a 10% reduction in yield. Increasing the reaction temperature improved the reaction rate at the expense of assay yield. Overall, the HATU-mediated cyclization proved to be a superior reagent in terms of reaction rate and yield. However, the combination of EDC/HOPO provided a cost-effective alternative to HATU. Consequently, we opted to examine *both* the HATU and EDC/HOPO leads in greater detail with particular emphasis placed on optimizing yield while reaction concentration was increased and purification and isolation strategies were identified.

HATU-Mediated Cyclization. With HATU, we first sought to understand the impact of dilution on reaction yield. Increasing reaction concentration above 20 g/L results in a precipitous drop in yield with concomitant formation of dimer, trimer, and additional higher order species. The best yields were obtained with substrate concentration below 10 g/L of substrate; however, little additional yield improvement was realized below 20 g/L. One potential strategy for overcoming this high dilution requirement would be to mimic the high dilution conditions by slow addition of the macrocycle precursor **16** to a concentrated solution of the coupling agent. For this to be successful, the rate of addition has to be equal to or less than the rate of macrocyclization to prevent buildup of **16**. Fortunately, the reaction with HATU is instantaneous at all temperatures examined. After careful examination of reaction rate, temperature, and concentration, we were pleased to find that reverse addition reactions run at final substrate concentration of 50 g/L instead of 10 g/L produced the desired macrocycle **9** in yields (70−75%) similar to the high dilution conditions with a nearly identical impurity profile.

EDC/HOPO Macrolactamization. It should be noted that HATU is a relatively expensive coupling agent. As noted

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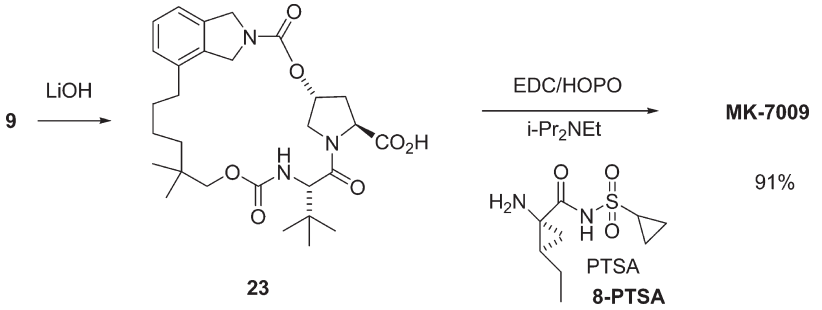
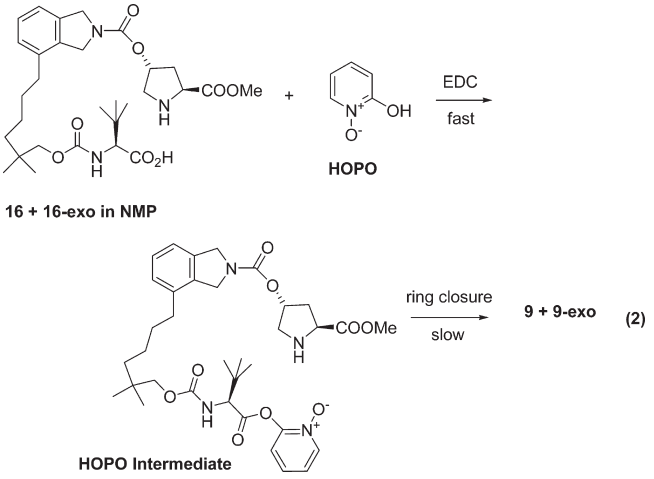
Scheme 6. Final Steps To Prepare MK-7009



above, we also identified EDC/HOPO as an effective surrogate for HATU. Analogous to the reaction with HATU, the EDC/HOPO-mediated cyclization is very sensitive to reaction concentration, with optimum yields obtained at or below 10 g/L. For EDC/HOPO, reactions were typically complete within 12 h. The rate-limiting step in this cyclization is likely ring closure of the proline amine with the HOPO-activated ester of the *tert*-butylglycine intermediate. This intermediate was observed by HPLC and NMR and was formed within ~30 min after addition of EDC to the reaction mixture (eq 2).



(2)

Efforts to expedite this cyclization were unsuccessful. Consequently, attempts to run this reaction using the reverse addition strategy gave lower yields depite extensive exploration. In order to maximize yields, we therefore ran the cyclization reaction at 10 g/L substrate in a 50/50 mixture of DMF/acetonitrile. Following reaction completion, the acetonitrile is removed under reduced pressure to produce a 20 g/L solution of **16**. Increasing the acetonitrile concentration beyond 50% resulted in an unacceptable loss of yield. Given the cost of HATU, we ultimately opted to use the EDC/HOPO reaction conditions to support ongoing trials.

Isolation of Macrocycle 9. Having identified a macrocylization reaction, emphasis shifted toward isolation of macrocyle **9** in acceptable purity. This would require removal of impurities generated during the Heck, hydrogenation, and cyclization reactions. Specifically, we were concerned about removal of regioisomeric impurities and dimeric/trimeric impurities, which would be difficult given their similarity to the desired product. After careful screening of conditions, it was determined that crystallization of **9** from the DMF reaction mixture could be effected by slow addition of water. This served to remove the majority of the palladium waste, HATU- or EDC/HOPO-derived byproduct, the remaining DCHA salt, and, most importantly, nearly all of the regioisomers (exomethyl isomer) that originated from the Heck reaction. Unfortunately, the dimer/trimeric/oligomeric species were not removed. Instead of carrying this mixture forward for downstream purification, these impurities were removed by redissolving the macrocycle **9** in isopropyl acetate with 5 mol equiv of water and subsequently crystallized by slow addition of methylcyclohexane to produce the macrocycle with <1% dimer/trimer/oligomeric impurities as the crystalline monohydrate. Thus, the 22-membered macrocycle **9** was obtained in sufficient purity after three steps and two crystallizations in 50−55% overall yield from intermediate **15**.

Final Coupling and API Isolation. Preparation of MK-7009 is illustrated in Scheme 6. Saponification of the ester intermediate (compound **9**) was straightforward, providing the desired acid in nearly quantitative yield (98% assay yield). Removal of color generated during the saponification process was accomplished by treatment with Darco KB-G (40 wt %). The acid was not isolated, the solvent was switched from IPAc (isopropyl acetate) to DMF prior to side chain coupling. Formation of MK-7009 is accomplished in 91% assay yield by treating a DMF/IPAc solution of the macrocyclic acid with the side chain **8**,[38,39] EDC, HOPO, and DIPEA. Following an aqueous workup, the isopropyl acetate was replaced with ethanol via constant volume distillation. Careful addition of aqueous potassium hydroxide produces the potassium salt, which crystallizes from solution. This crystalline potassium salt is a nonstoichiometric hydrate and the water content depends on humidity of the storage conditions. The drying process also needs to run under nitrogen with controlled humidity. Alternatively, the neutral MK-7009 can be isolated from IPAc−heptane as a heptane solvate, which upon drying turns into anhydrous crystalline solid. Both forms were used in clinical formulations.

Additional Avenues for Route Optimization: Sonogashira Route. With a robust process in hand to support preparation of MK-7009 for clinical needs, we again shifted our attention to improving the overall yield of the macrolactamization route. In particular, we focused on identifying alternatives to the Heck reaction, as it resulted in a 13% yield loss due to regioisomer formation. We addressed this challenge by focusing on the use of a Sonogashira-type coupling, which would remediate the regioisomer issue.[40−42] The preparation of the alkyne linker is illustrated in Scheme 7 and relies upon a key "zipper" reaction to convert an internal alkyne **24** to the required terminal alkyne **25**. Subsequent carbamate formation with CDI and *tert*-butylglycine followed by treatment with DCHA yields the salt of

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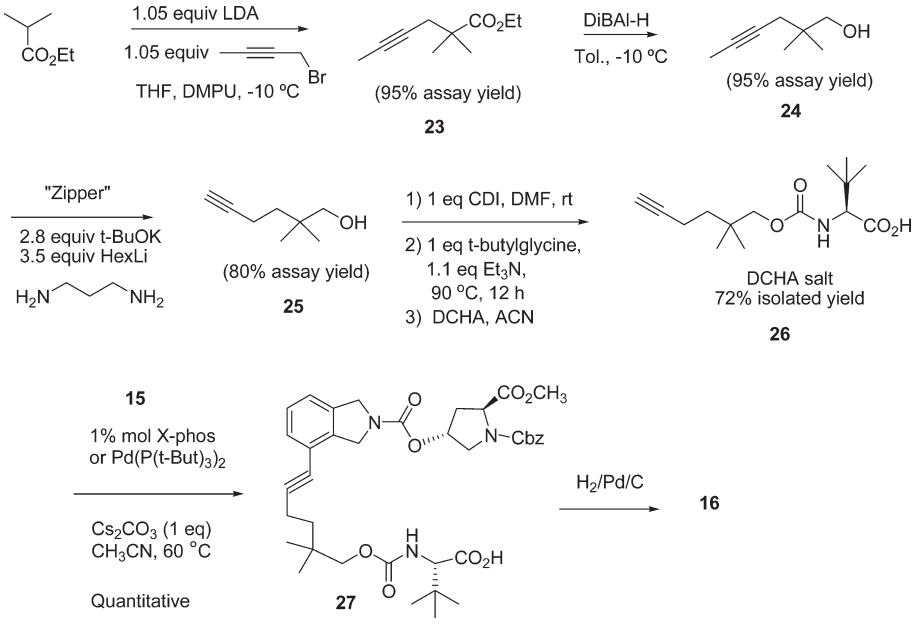
Scheme 7. Sonogashira and Hydrogenation Sequence To Produce Premacrocycle 16



alkyne linker **26**. The Sonogashira reaction of **26** and aryl bromide **15** was mediated by the X-Phos/Pd catalyst.[43] In contrast to the Heck reaction, this Sonogashira coupling step proceeds in near quantitative yield without production of multiple isomers. Subsequent hydrogenation of this coupling product yields intermediate **16**, which converges with the current macrolactamization route.[44]

The overall relative yield of the Sonogashira through macrolactimization is 10−15% higher than the Heck route due to the absence of regioisomers generated in the Heck reaction. However, the starting material cost required for the alkyne synthesis is substantially higher due to the added cost of 1-bromo-2-butyne and butyllithium. While the Heck reaction produces 13% of the undesired regioisomer, it employs ligandless conditions with palladium, while the Sonogashira reaction requires use of a relatively expensive ligand. In addition, this route requires one additional synthetic transformation. Taken together, the Heck route was viewed as both more robust and cost-effective than the Sonogashira route to MK-7009 at this stage in our development. Nonetheless, the Sonogashira route will be attractive if an alternate synthesis of the alkyne precursor is identified in concert with a more cost-effective Sonogashira coupling reaction.

SUMMARY AND CONCLUSIONS

The primary challenges to developing a scalable, cost-effective synthesis for MK-7009 are identifying an efficient ring-closing strategy *and* a concise synthesis of the intermediates needed to construct the macrocycle precursors. The choice of Heck reaction followed by macrolactamization was based on the overall efficiency and cost of the process for all the steps. Taken together, we were able to develop a scalable route to MK-7009 in ca. 20% overall yield to support clinical trials.

EXPERIMENTAL SECTION

General. Chemical reagents were used as received from suppliers except otherwise noted. NMR spectra were taken on 400, 500, or 600 MHz spectrometers. Low-resolution mass spectra were taken by LC−MS with acetonitrile and ammonium formate buffer and the mass spectrometer set for positive electron ionization. The weight purity of isolated compound or crude solution may be analyzed against a working standard and reported as weight percent. The purity or reaction conversion based on HPLC peak integration may also be reported as area percent (A%).

3-Bromo-*N*-benzylisoindoline pTSA Salt (18-PTSA). 3-Bromo-*O*-xylene (17.1 g, 94.6 mmol) and *N*-bromosuccinimide (36.1 g, 208.1 mmol) were charged to a round-bottom flask followed by chlorobenzene (194 g) and benzoyl peroxide (131.3 mg, 0.38 mmol). The mixture was stirred and warmed to 88−90 °C for 1.5 h and another charge of benzoyl peroxide (131.3 mg, 0.38 mmol) was made. The mixture was stirred at 88−90 °C for a further 2 h, and HPLC indicated complete reaction. The batch was cooled to 25 °C and washed with water (3 × 75 mL), giving the dibrominated product **17** (estimated 23.8 g, 75% yield) as a solution in chlorobenzene. The chlorobenzene solution was diluted with toluene (147 g) and water (3.0 mL) added. Solid sodium bicarbonate (14.2 g, 173.8 mmol) was charged to the vessel followed by benzylamine (10.0 g, 93.7 mmol). The reaction was heated to 93−95 °C for 1.5 h, when HPLC indicated complete reaction (Caution: carbon dioxide off gas!). The batch was cooled to 20 °C and washed with water (140 g) and then the organic layer extracted in to 1 M phosphoric acid (3 × 100 g). The lower aqueous extracts were combined and returned to the clean vessel. *p*-Toluenesulfonic acid monohydrate (2.4 g) was charged as a solid to the batch and the mixture seeded. The batch was stirred at 20 °C for 20 min for seed bed to form before adding the remaining *p*-toluenesulfonic acid monohydrate (9.6 g, 63.1 mmol total, 1.2 equiv based on assay) in one portion. The mixture was stirred at 20 °C for 1 h before filtering. The solid was washed with water (50 g), collected on trays, and dried in vacuum at 40 °C for 3 days. 3-Bromo-*N*-benzylisoindoline pTSA salt **18-PTSA** (19.98 g) was isolated as an off-white solid in 47% yield from 3-bromo-*o*-xylene, 99% pure based on HPLC analysis. Mp 187−189 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.64 (m, 2H), 7.60−7.58 (om, 2H), 7.55 (m, 1H), 7.52−7.49 (om, 3H), 7.35−7.29 (om, 2H), 7.19 (m, 2H), 4.78 (s, 2H), 4.68 (s, 2H), 4.65 (s, 2H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 143.6, 141.8, 137.1, 135.7, 133.30, 132.4, 131.7, 131.5, 130.7, 130.0, 127.0, 123.5, 118.0, 60.4, 59.6, 21.4; EIMS M + 1 = 288, 290 calcd for $C_{15}H_{14}BrN$ = 287, 289.

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Anal. Calcd for $C_{22}H_{22}BrNO_3S$: C, 57.39; H, 4.82; N, 3.04; S, 6.96. Found: C, 57.53; H, 4.70, N, 2.97, S, 6.70.

3-Bromoisoindoline HCl Salt (19). 3-Bromo-*N*-benzylisoindoline pTSA salt **18-PTSA** (40.5 g, 87.9 mmol) was charged to a 500 mL round-bottom flask followed by chlorobenzene (179 g). Sodium hydroxide solution (1 M, 200 mL) was charged to the flask in one portion and the mixture stirred vigorously for 15 min until all the solids had dissolved. The layers were separated, and the bottom organic layer was washed with water (132 g). Once again the layers were separated, and the bottom organic layer was azeotropically dried by distilling chlorobenzene (20 mL) from the mixture under reduced pressure (KF of batch = 51 μg/mL).

1-Chloroethylchloroformate (ACE-Cl, 16.3 g, 114 mmol) was added to the above solution keeping the batch temperature <15 °C. The batch was warmed to 90−95 °C and stirred at this temperature for 2 h. HPLC analysis indicated 8.8% starting material remaining. More 1-chloroethylchloroformate (1.14 g, total 122 mmol) was added to the reaction and the batch heated to 90−95 °C overnight (14 h). HPLC analysis indicated the reaction was complete, with <2% starting material remaining. The batch was cooled to 40 °C and methanol (32 g) charged to the flask. The mixture was heated to 60−65 °C and kept at this temperature for 4 h. HPLC analysis indicated the reaction was complete, with <1% carbamate intermediate remaining. The batch was cooled to 20 °C and aged for 1 h before filtering. The solid was washed with acetonitrile (76 g), collected on trays, and dried in vacuo at 40 °C for 4 days. 3-Bromoisoindoline HCl salt **19** (18.4 g) was isolated as a white solid in 89% yield, 98.2% pure by weight based on HPLC analysis. Mp 326−328 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (br s, 2H), 7.58 (d, J = 7.8 Hz, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 4.62 (s, 2H), 4.47 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.4, 135.5, 131.0, 130.6, 122.3, 116.30, 51.0, 51.0; EIMS M + 1 = 198, 200, calcd for C_8H_8BrN = 197, 199. Anal. Calcd for $C_8H_8BrNHCl$: C, 40.97; H, 3.87; N, 5.97. Found C, 41.16; H, 3.63, N, 5.86.

CBZ Carbamate Bromide (15). To a 3 L, four-neck round-bottom flask, with mechanical stirrer, thermocouple, and nitrogen/vacuum line, was charged DMF (190 mL), *N*-Cbz-L-hydroxyproline methyl ester (54.3 g, 194.5 mmol) and 1,1′-carbonyl diimidazole (30.4 g, 187.5 mmol). The reaction was inerted with vacuum/nitrogen cycle and heated to 65−70 °C. After 1 h, HPLC analysis showed 97% conversion to the intermediate. Bromoindoline HCl **19** (41.4 g, 176.6 mmol) was added to the reaction mixture and aging at 65−70 °C continued. The batch changed from slurry to solution as the reaction proceeded. After 6 h HPLC analysis showed >99% conversion. Then 190 mL of MeCN and 380 mL of water were added sequentially to crystallize the product. The mixture was seeded after 60 mL of water and aged to develop a seed bed. The water addition after seeding was done very slowly in order to allow a good seed bed to form. During the addition the slurry became very thick and vigorous stirring was necessary. The solid was isolated by filtration. The cake was washed with 1:1 DMF/water (200 mL) and then water (2 × 200 mL). The cake was dried on the filter under nitrogen and then for 6 h in the vacuum oven at 50 °C. The product was obtained as a pale pink solid (80.60 g) in 91% yield. HPLC showed >95% by area; chiral assay >99% ee; mp 99−100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) mixture of rotamers δ 7.42 (d, J = 7.7 Hz, 2H), 7.37−7.31 (om, 4H), 7.27 (m, 1H), 7.22−7.15 (om, 2H), 5.35 (m, 1H), 5.24 (dd, J = 12.4, 7.1 Hz, 0.53H), 5.20 (d, J = 12.4 Hz, 0.47H), 5.14 (dd, J = 12.4, 5.3 Hz, 0.53H), 5.07 (d, J = 12.4 Hz, 0.47H), 4.81 (br s, 1H), 4.73−4.68 (om, 2H), 3.91−3.77 (om, 1H), 3.79$_0$ and 3.786 (2s, 1.6H), 3.57$_1$ and 3.56$_7$ (2s, 1.4H), 2.57−2.47 (om, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) ∼1:1:1:1 mixture of rotamers δ 172.82, 172.78, 172.65, 172.60, 155.05, 155.01, 154.40, 153.88, 153.80, 138.44, 138.29, 138.23, 137.57, 137.44, 137.40, 136.50, 136.42, 130.78, 130.74, 129.65, 129.61, 129.58, 128.61, 128.60, 128.23, 128.22, 128.07, 121.73, 121.70, 121.58, 121.55, 117.77, 117.62, 117.58, 73.85, 73.81, 73.16, 73.09, 67.50, 58.22, 58.19, 57.97, 57.94, 54.05, 53.62, 53.51, 53,.16, 53.11, 52.83, 52.73, 52.62, 52.36, 37.24, 37.14, 36.18, 36.09; $[\alpha]_{589}^{25}$ = −46 (1 g/100 mL MeOH); EIMS M + 1 = 503, 505, calcd for $C_{23}H_{23}BrN_2O_6$ = 502, 504. Anal. Calcd for $C_{23}H_{23}BrN_2O_6$: C, 54.88; H, 4.61; N, 5.57. Found C, 54.99, H, 4.37, N, 5.47.

2,2-Dimethyl-5-hexenol (21). *Alkylation.* A 50 mL round-bottom flask was charged with THF (10 mL) and diisopropylamine (3.25 mL, 2.35 g, 23.2 mmol) and the solution cooled to around −20 °C. Hexyllithium (2.3 M in hexane, 9.65 mL, 22.2 mmol) was added over 30 min at −20 to −10 °C, and the batch was aged for an additional 15 min. Ethyl isobutyrate (2.46 g, 21.1 mmol) was added over 15−30 min while the batch temperature was kept between −10 to −20 °C. At the end of the addition, DMPU (1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone, 2.53 mL, 21.1 mmol) was added over a few minutes, and the resulting solution was aged at −10 to −20 °C for 15 min. 4-Bromo-1-butene (3.0 g, 22.2 mmol) was then added dropwise over 15−30 min while keeping the batch temperature around −20 °C. The resulting thin slurry was aged for 30 min at −20 °C, allowed to warm to rt, aged for another hour, and quenched by MTBE (21 mL) and aqueous HCl (1.5 N, 17 L, 25.3 mmol). Layers were separated, and the organic layer was washed with water (2 × 17 mL) and concentrated to an oil (3.31 g assay, 92% yield of **20**), which was dissolved in toluene (6 mL), and concentrated again to give 4.85 g of crude liquid product **20** (65 wt %, 3.16 g assay, 88% isolated yield), which was used in the next step without further purification (Caution: the product is volatile; do not overdistill). ^{1}H NMR spectroscopy was used to confirm the identity of intermediate **20**.[3]

DiBAL-H Reduction. A round-bottom flask was charged with a 1 M THF solution of diisobutylaluminum hydride (DiBAL-H, 30.7 mL, 30.7 mmol) and was cooled to around −20 °C. Crude ester **20** (2.9 g, 75 wt %, 2.18 g assay, 12.8 mmol) was added over 30 min and the temperature was kept around −10 °C. The batch was aged for 30 min and was transferred over 30 min into a biphasic solution made of MTBE (22 mL) and a 1.5 M aqueous Rochelle's salts solution at 0 °C. The resulting mixture was aged at 5−10 °C for 1 h, allowed to warm to rt, and aged 2 h. Layers were separated, and the organic layer was washed with 1 N aqueous HCl (17 mL, 17 mmol) and with water (2 × 17 mL) and concentrated to give 3.2 g of crude oily product **21** (51 wt %, 1.64 g assay, 100% assay yield), which was used in the next step without further purification. NMR spectroscopy was used to confirm the identity of the material.[3]

Linker DCHA Salt (5-DCHA). A 50 mL round-bottom flask was charged with DMF (13 mL) and the crude alcohol **21** (3.2 g, 51 wt %, 1.64 wt %, 12.8 mmol), and the solution was cooled to around 5 °C. CDI (1,1′-carbonyldiimidazole, 2.67 g, 16.5 mmol) was added portionwise over 15 min. The resulting homogeneous mixture was aged at rt for 30 min. A first portion of CDI (2.08 g, 12.8 mmol) was added, and the reaction was checked by ^{1}H NMR (CH_2O CDI adduct/CH_2OH δ 4.1 and 3.25 ppm). More CDI was added until the δ 3.25 ppm peak disappeared. The reaction was exothermic and the temperature rose to 20−30 °C over 15 min. L-*tert*-Leucine [(*S*)-*tert*-butylglycine, 2.16 g, 16.5 mmol] was added to the reaction mixture in one portion followed by the addition of triethylamine (2.5 mL, 17.9 mmol). The resulting slurry was heated to 90 °C for 12 h and allowed to cool to rt. The slurry turned homogeneous at 90 °C upon aging. The solution was partitioned between MTBE (15 mL) and a 0.5 N aqueous NaOH solution (19 mL). Layers were separated, and the organic phase was discarded. To the DMF aqueous basic layer was added MTBE (24 mL) and it was neutralized to pH∼1−2 with 6 N aqueous HCl solution (ca. 11 mL). Layers were separated, and the organic layer was washed with with water (2 × 15 mL). The organic solution was concentrated, switched to acetonitrile (ca. 50 mL final, typically KF ∼ 500 ppm), and heated to 45 °C. Dicyclohexylamine (2.32 g, 128 mmol) was added over 1 h. The salt crystallized, the slurry was aged at 45 °C for 6 h, and the slurry was allowed to cool to rt, aged 1−2 h, filtered, and rinsed with acetonitrile

(10 mL). The resulting white solid **5-DCHA** was dried at 40 °C in an oven for 48 h to give 5.1 g of product (85% overall yield). HPLC analysis >90% by area; chiral HPLC analysis 99% ee; mp 108−110 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 5.81 (ddt, J = 17.0, 10.4, 6.7 Hz, 1H), 4.99 (dt, J = 17.0, 1.6 Hz, 1H), 4.90 (m, 1H), 3.88 (s, 1H), 3.82 (d, J = 10.5 Hz, 1H), 3.73 (d, J = 10.5 Hz, 1H), 3.17 (dt, J = 11.3, 4.0 Hz, 2H), 2.08−2.01 (om, 6H), 1.87 (m, 4H), 1.72 (m, 2H), 1.43−1.29 (om, 10H), 1.22 (m, 2H), 0.98 (s, 9H), 0.93_3 (s, 3H), 0.92_9 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.9, 158.7, 140.6, 114.6, 73.7, 66.4, 54.6, 39.7, 35.4, 35.1, 30.8, 29.6, 27.7, 26.3, 25.7, 24.8, 24.7. $[\alpha]^{25}_{589} = -7.22$ (1 g/100 mL MeOH); EIMS M + 1 = 286, calcd for $C_{15}H_{27}NO_4$ = 285. Anal. Calcd for $C_{27}H_{50}N_2O_4$: C, 69.49; H, 10.80; N, 6.00. Found C, 69.49; H, 10.84; N, 5.98

Heck Reaction To Produce 22 and 22-exo. A three-neck 1000 mL round-bottom flask was charged with bromide **15** (50.0 g, 99.3 mmol), **5-DCHA** (55.6 g, 120 mmol, 1.2 equiv), NMP (75 mL). The heterogeneous mixture was stirred and warmed to 45 °C. Upon dissolution of all solids, the red-orange solution was subjected to nitrogen−vacuum purge cycles and placed under nitrogen (Degassing at room temperature results in formation of an intractable foam). The catalyst, palladium acetate (0.223 g, 1.0 mmol), was weighed out in the air and quickly transferred to the reaction flask. After the catalyst charge, the solution was subjected to three nitrogen−vacuum purge cycles, placed under nitrogen, and warmed to 100 °C. The reaction solution darkens over time, after which a black precipitate forms. Qualitatively, this typically coincided with the end of the reaction (1.5 h). After 2 h, the solution was sampled and judged to be complete by HPLC (>99% conversion by area). Upon cooling to rt, the heterogeneous reaction was charged with BHT (butylated hydroxyltoluene, 0.25 g, 1.1 mmol, to inhibit air oxidation) and transferred to the hydrogenation vessel. The original reaction vessel was rinsed with NMP (10 mL × 2) and the NMP was combined with the reaction solution in the hydrogenation vessel. White solid (DCHA·HBr salt) is present in the mixture.

Hydrogenation To Produce 16 and 16-exo. To the solution of Heck product **22 and 22-exo** (97.9 mmol, in NMP as described in the previous step) was added dry palladium on carbon (Johnson Matthey, dry 10%, 2.5 g). The solution was transferred to a hydrogenation vessel, subjected to five vacuum−nitrogen purge cycles, and placed under hydrogen gas (40 psig) at room temperature. After overnight aging, the solution was vented to atmospheric pressure and placed under nitrogen. The black solution was filtered through a thin layer of Solka Floc and the filter cake was rinsed with DMF (1000 mL). The DMF/NMP solution of product was carried on to the macrocyclization. HPLC indicated the ratio of the **16** to **16-exo** product was typically 88:12. The final target concentration after filtration and DMF rinse was approximately 0.064 mmol/g.

Macrocyclization Reaction with EDC/HOPO To Produce Macrocyclic Ester 9 and 9-exo. At 20 °C, the DMF/NMP solution of hydrogenation product **16** and **16-exo** (98 mmol total isomers in 88/12 ratio in 1.505 kg solution) was added to a 12 L flask containing HOPO (14.5 g, 130.4 mmol). DMF (1.5 L) and acetonitrile (2.8 L) were added. After 10 min, DIPEA (diidopropylethylamine, 30.3 mL, 174.0 mmol) was added to the stirred solution followed by EDC·HCl (33.3 g, 174.0 mmol). The mixture was stirred at room temperature overnight. The reaction was judged complete by HPLC (>99% conversion). During the aging, some DCHA·HCl crystallized from solution. HPLC indicated that the macrocyclic ester concentration was 7.5 mg/g. The batch was concentrated to remove acetonitrile on a Rotovap (~20 Torr vacuum and bath temp at 50 °C). Concentration was judged complete when distillate condensation halts. HPLC indicated that the macrocyclic ester concentration was 13.2 mg/g. NMR indicated that 3 vol % acetonitrile remained, and the batch weighed 2.83 kg. The solution was then filtered through a thin layer of Solka Floc to remove DCHA·HCl. To the solution was added water (850 g, 30% total charge) over 30 min, and the solution was kept at 22 °C. The batch was seeded with 0.35 g of crystalline product and aged for 0.5 h for seed bed to form. Additional water (1.95 kg, 70% total charge) was added to the batch over 5 h. The batch was allowed to stir overnight (16 h). The slurry was filtered and the filter cake was rinsed with water (500 g × 3). The resulting wet cake was dried under vacuum with nitrogen sweep for 20 h. Crude **9** was obtained as a light tan solid (53.6 g, 67 wt %, 66% isolated yield for this step, 61% from Heck step). Typically by HPLC analysis, macrocyclic ester **9** was 95%, dimer 4%, exomethyl isomer 0.5%. Two diasteromers derived from either (*R*)-*tert*-butylglycine or from (2*S*,4*S*)-hydroxyproline were synthesized independently and shown by HPLC not to be present in the reaction mixture.

Purification of Macrocyclic Ester 9. The crude ester **9** (25.3 g, 67 wt %) was slurried in IPAc (280 mL) and warmed to 45 °C. After 15 min, the homogeneous solution was mixed with DARCO-KB-G (1.6 g). After 40 min, the mixture was filtered through a thin layer of Solka Flock. The filter cake was rinsed with IPAc (33 mL × 3). Note that the third rinse with IPAc contained negligible quantities of macrocycle **9**. Quantitative assay indicated that the loss to the carbon treatment is <5%. The combined IPAc layers were concentrated to 189 mg of **9**/g of solution. The solution was warmed to 50 °C, and water (1.25 mL, 2.25 equiv) was added. After 10 min, previously generated seed (85 mg, 0.5 wt %) was added to yield a thin crystalline seed bed. After 30 min, methylcyclohexane (470 mL) was added over 1.5 h and the batch was allowed to cool to room temperature over approximately 2 h. After an overnight age, the solution was filtered and the light brown filter cake was washed with MeCy/IPAc (98:2 MeCy/IPAc 50 mL) and dried overnight under vacuum with a nitrogen sweep. The title compound was obtained as light brown solid (15.2 g, 96 wt %, water content 3%, 87% recovery). Typical HPLC area 98% with <0.9% dimer, 0.1% exomethyl isomer. Typical palladium level was 100 ppm. Overall yield (Heck, hydrogenation, macrocyclization, recrystallization) to macrocyclic ester **9** hydrate was 53.5%. This yield takes into account measured samples taken for analysis and front runs. Mp 160−162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 7.5 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 5.53 (d, J = 9.7 Hz, 1H), 5.32 (t, J = 3.8 Hz, 1H), 4.72 (AB doublet, J = 15.0 Hz, 2H), 4.63 (dd, J = 10.3, 7.7 Hz, 1H), 4.52 (AB doublet, J = 15.3 Hz, 2H), 4.42 (t, J = 9.4 Hz, 2H), 4.15 (dd, J = 11.7, 1.7 Hz, 1H), 3.81 (dd, J = 11.7, 3.8 Hz, 1H), 3.76 (s, 3H), 3.24 (d, J = 10.7 Hz, 1H), 2.74 (ddd, J = 14.4, 7.6, 1.4 Hz, 1H), 2.52 (ddd, J = 13.3, 10.1, 6.2 Hz, 1H), 2.41 (ddd, J = 13.3, 10.7, 6.2 Hz, 1H), 2.15 (ddd, J = 14.3, 10.5, 3.9 Hz, 1H), 1.77 (s, 1H), 1.49 (m, 1H), 1.34−1.25 (om, 3H), 1.16 (m, 1H), 1.06 (s, 9H), 0.96 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 171.0, 156.5, 153.8, 137.8, 136.4, 135.2, 128.2, 127.8, 120.3, 74.4, 72.7, 59.4, 57.8, 54.3, 52.8, 52.5, 51.1, 37.1, 36.8, 35.7, 34.3, 33.8, 30.9, 26.4, 25.3, 24.0, 23.6; $[\alpha]^{25}_{589} = -64.05$ (0.5 g/100 mL MeOH); EIMS M + 1 = 558, calcd for $C_{30}H_{43}N_3O_7$ = 557. Anal. Calcd for $C_{30}H_{43}N_3O_7H_2O$: C, 62.59; H, 7.88; N, 7.30. Found C, 62.73; H, 7.77; N, 7.32.

Macrocyclization via Reverse Addition To Produce Macrocyclic Ester 9 and 9-exo. HATU (9.93 g, 26.1 mmol) followed by DMF (147 g) were charged to a 500 mL round-bottom flask. The mixture was stirred to dissolve the solid and then cooled to 0 °C. The compound **16** DMF solution (from 21.3 mmol aryl bromide Heck reaction followed by hydrogenation, 12.1 g estimated desired isomer) was mixed in another 500 mL flask together with DIPEA (12.8 g, 94.6 mmol) at <30 °C. This basic solution was slowly added to the HATU/DMF solution over 2 h at 0 °C and the mixture stirred at 0 °C for an additional 30 min. HPLC indicated <0.5% starting amino acid intermediate remaining. The batch was warmed to 20 °C. Water (80 g total) was charged to the batch and the temperature was kept at 20−25 °C, producing a cloudy mixture. The batch was seeded and stirred for 20 min before resuming the water addition (162 g over 2 h). The batch was stirred at 20 °C overnight and filtered, and the cake was washed with water twice (25 g each). The solid was dried under nitrogen on the filter for 3 days and then transferred to the oven and dried in

vacuo at 45 °C for 2 days. The crude macrocylic ester (11.0 g) was isolated as an off-white solid 75.3% by weight and 95% by area in 65% overall yield from the Heck step (72% for cyclization step).

Ester Saponification To Produce 23. A 100 mL round-bottom flask equipped with an overhead stirrer and thermocouple was charged with a solution of the macrocylic ester **9** (1.78 g) in THF (9.6 mL) and cooled to 5 °C. An aqueous solution of lithium hydroxide (1 N, 9.6 mL, 9.6 mmol) was added slowly via addition funnel over 30 min while the batch temperature was kept below 15 °C. With the same addition funnel, methanol (1.8 mL) was added over 10 min at 15 °C, after which the white, heterogeneous mixture was allowed to warm to room temperature. Upon warming, the solution became homogeneous. After ca. 30 min, the solution turned from light yellow to dark brown. The reaction, sampled at this time, was judged complete by HPLC analysis (>99.9A% conversion). Concentration of the acid **23** was 85.2 mg/g . The batch was cooled to 5 °C and 1 N HCl (11.2 mL) added. The batch was warmed to 20 °C and diluted with IPAc (18 mL, 10 vol). After agitating for 15 min, the layers were separated, and the organic layer was collected (HPLC assay showed 1.7 g macrocyclic acid, 98% assay yield). The IPAc solution was treated with 0.69 g of Darco KB-G (40 wt %) at 20 °C for 10 min, and the solution was filtered through Solka Floc. The IPAc solution was concentrated under reduced pressure, with the temperature was kept below 25 °C, to 10 mL and flushed with 10 mL of IPAc. The solution was diluted with DMF (8 mL) and the concentration was continued until the final batch volume was 8 mL. The batch was diluted with DMF (2 mL) and IPAc (8 mL) to approximately 91.7 mg/g **23** concentration.

Preparation of Side Chain 8-PTSA Salt.[45]. *Hydrogenation.* Compound **24** (12.7 g, 364 mmol, >99% ee) was charged to a hydrogenation vessel, followed by 1.3 g of 5% ruthenium on carbon catalyst. The hydrogenation vessel was then charged with 60 g of methanol (KF < 400 ppm) and pressured to 50 psi with hydrogen, and the batch was aged for 5.5 h at 20−25 °C. The end of reaction sample revealed that the desired conversion was reached (<2% starting material). The batch was then filtered through Solk Floc and the cake washed with more methanol. The combined solution was vacuum concentrated to about 80 mL and solvent switched to 2-propanol with addition of 160 g of 2-propanol. The final volume of product solution was 150 mL. This solution was used in the next step. The assay yield of **25** is ca. 90%. Chiral HPLC showed >99% ee.

*Boc Removal To Produce Side Chain **8-PTSA**.* To the product solution in 2-propanol from the hydrogenation step was added 8.8 g of *p*-toluenesulfonic acid monohydrate (PTSA, 463 mmol), with 15 g of 2-propanol flush. The batch was then heated to 63−67 °C over ∼1 h to control off gassing and aged for 6 h at 63−67 °C for complete reaction. The batch was then cooled to 45 °C and 37.1 g of *n*-heptane was charged while the batch was maintained at 42−46 °C. A slurry formed. The batch was cooled to 15−25 °C, aged for 1 h, and then filtered, and the filtrate was collected (159.8 g) before washing the cake with 30 g of *n*-heptane at 15−25 °C. The cake wash (54.1 g) was collected into a separate container, and the wet cake was blown dry with nitrogen at 15−25 °C. The dry cake of the title compound was collected as a white crystalline solid (11.2 g, 75% yield for the hydrogenation and Boc removal). The solid has <0.5 wt % residue solvent, 99.8% ee, 99.3 wt %, and 99.9 A% by HPLC. Mp 218−220 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.71 (m, 2H), 7.24 (m, 2H), 3.06 (m, 1H), 2.37 (s, 3H), 1.84 (t, J = 7.5 Hz, 1H), 1.70−1.58 (om, 2H), 1.57−1.48 (om, 2H), 1.31 (m, 1H), 1.23 (m, 1H), 1.17−1.07 (om, 2H), 1.04 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 169.6, 143.6, 141.9, 130.0, 127.1, 41.7, 32.3, 30.1, 21.6, 21.4, 17.6, 13.5, 6.8, 6.7; $[\alpha]_{589}^{25}$ = −54.15 (1 g/100 mL MeOH); EIMS M + 1 = 233, calcd for $C_9H_{16}N_zO_3S$ = 232. Anal. Calcd for for $C_{16}H_{24}N_2O_6S_2$: C, 47.51; H, 5.98; N, 6.93; S, 15.85. Found C, 47.36; H, 5.66; N, 6.83; S, 15.53.

Preparation of MK-7009. A 500 mL round-bottom flask equipped with an overhead stirrer, nitrogen inlet, and thermocouple was charged with macrocylic acid **23** in DMF and IPAc (5.37 g in 27 mL of IPAc and 27 mL of DMF). The solution was set stirring and **8-PTSA** (4.39 g, 3.46 mmol) was added as a solid. Upon dissolution (<10 min), hydroxylpyridine *N*-oxide (HOPO, 1.21 g, 3.21 mmol) was added as a solid. The batch was cooled to 15 °C and DIPEA (2.68 g, 2.08 mmol) was added via addition funnel while the temperature was maintained below 20 °C. Solid EDC·HCl (2.65 g, 1.38 mmol, 1.4 equiv) was added. After 3 h, the reaction was judged complete by HPLC (>99.8 A% conversion, 94% assay yield, 7.01 g). The batch was transferred to a 500 mL separation funnel and diluted with IPAc (100 mL) and water (100 mL). After the first cut, the organic layer was washed with 50 mL of 1 M HCl, water (50 mL), and brine (50 mL). The IPAc solution was concentrated and flushed with ethanol (50 mL) until there was 2.5 mol % IPAc in ethanol, as judged by ^{1}H NMR spectroscopy. HPLC assay indicated 6.86 g of MK-7009 (92% yield).

MK-7009 Neutral Isolation. MK-7009 (11 g by assay) in 50 mL of wet IPAc (0.37% water) was added simultaneously with 63 mL of heptane to a seed bed of MK-7009 (1.2 g) in 24 mL of 57/43 v/v heptane/IPAc at 50 °C over a 12 h period. High seed loading was used to ensure high-quality crystal growth and consistent, high purity of the isolated product. An additional 31 mL of heptane ws added over 3 h, bringing the solvent composition to 65/35 (v/v) heptane/IPAc. The batch is then cooled over 3 h back to 20 °C. The batch was filtered and the filter cake washed with 22 mL of 65/35 (v/v) heptane/IPAc and then washed twice more with 22 mL of heptane each. The recovered wet cake was a heptane solvate. The cake was dried under vacuum with nitrogen sweep at 70 °C to generate pure MK-7009.[3] The typical yield is 90−95%. Mp 175−177 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 7.5 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 6.85 (s, 1H), 5.55 (d, J = 9.7 Hz, 1H), 5.32 (t, J = 3.4 Hz, 1H), 4.74 (d, J = 14.6 Hz, 1H), 4.68 (d, J = 14.6 Hz, 1H), 4.54 (d, J = 14.7 Hz, 1H), 4.47 (d, J = 14.7 Hz, 1H), 4.45−4.43 (om, 2H), 4.35 (dd, J = 10.5, 6.8 Hz, 1H), 4.16 (d, J = 11.7 Hz, 1H), 3.85 (dd, J = 11.7, 3.4 Hz, 1H), 3.26 (d, J = 10.7 Hz, 1H), 2.91 (tt, J = 8.0, 4.8 Hz, 1H), 2.61 (dd, J = 14.2, 6.8 Hz, 1H), 2.51 (m, 1H), 2.40 (m, 1H), 2.30 (ddd, J = 14.2, 10.7, 3.4 Hz, 1H), 1.69 (dd, J = 8.3, 5.4 Hz, 1H), 1.63 (m, 1H), 1.55 (m, 1H), 1.48 (m, 2H), 1.41 (m, 1H), 1.37, m (1H), 1.34−1.24 (om, 5H), 1.15 (m, 1H), 1.05 (s, 9H), 1.03 (m, 2H), 0.96 (s, 3H), 0.95 (t, J = 7.4 Hz, 3H), 0.79 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 172.0, 169.6, 156.6, 153.7, 137.8, 136.3, 135.0, 128.3, 127.9, 120.4, 74.4, 73.0, 59.6, 59.3, 54.8, 52.7, 51.1, 39.9, 37.1, 36.7, 35.8, 35.1, 34.4, 33.8, 31.5, 30.9, 26.6, 25.2, 24.0, 23.6, 23.1, 20.0, 13.8, 6.4, 6.1; $[\alpha]_{589}^{25}$ = −40.84 (1.4 g/100 mL MeOH). Anal. Calcd for $C_{38}H_{55}N_5O_9S$ C, 60.22; H, 7.31; N, 9.24; S, 4.23. Found C, 59.82; H, 6.92; N, 9.16; S, 4.29.

MK-7009 K Salt Formation. To 75 mL round-bottom flask was added MK-7009 solution (2.037 g in 18 g total) in ethanol. The batch was heated to 50 °C and potassium hydroxide solution in ethanol−water was added slowly (893 mg, 17.8 wt % aqueous KOH mixed with 6.7 mL of ethanol, 1.05 equiv). Seed was added after 1 mL of the KOH solution was charged. The rest of the KOH solution was added over 30 min after aging the seed bed for 20 min. The addition funnel was rinsed with 2 mL of ethanol. The batch was stirred at 50 °C for 1 h and then cooled to rt over 3 h and stirred overnight. The solid was collected by filtration and washed with 3 mL of ethanol and dried on the filter via suction under 26−29% relative humidity (>15% RH required to produce the right crystal form). Alternatively, the drying process can be carried out at 50 °C with humid nitrogen sweep (humidity 15−50% relative humidity). MK-7009 potassium salt was obtained as off white crystalline solid (2.16 g). HPLC analysis showed 99 A%, 93% wt K salt (91% yield after correcting for purity and the seed, also present was water 4.3%, ethanol 0.8%, dimer 0.4%).[3] ^{1}H NMR (600 MHz, DMSO-d_6) (MK-7009 K salt exists as an approximate 87:13 ratio of amide bond rotamers, only data for the major

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rotamer are reported) δ 7.94 (s, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 7.08 (d, J = 7.6 Hz, 1H), 6.90 (d, J = 9.4 Hz, 1H), 5.15 (t, J = 3.8 Hz, 1H), 4.61 (s, (2H), 4.48 (s, 2H), 4.32 (dd, J = 9.4, 7.9 Hz, 1H), 4.27 (d, J = 9.4 Hz, 1H), 4.22 (d, J = 10.6 Hz, 1H), 3.93 (d, J = 12.1 Hz, 1H), 3.77 (dd, J = 12.1, 3.4 Hz, 1H), 3.21 (d, J = 10.6 Hz, 1H), 2.72 (m, 1H), 2.51−2.36 (om, 3H), 2.15 (m, 1H), 1.58−1.47 (om, 2H), 1.44 (m, 2H), 1.27−1.21 (om, 3H), 1.19 (dd, J = 7.6, 4.2 Hz, 1H), 1.11 (m, 1H), 1.07 (m, 1H), 0.95 (s, 9H), 0.93 (s, 3H), 0.88 (t, J = 7.6 Hz, 3H), 0.80 (dd, J = 9.4, 4.2 Hz, 1H), 0.75 (m, 1H), 0.60 (m, 1H), 0.73 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.8, 170.4, 169.4, 156.2, 153.3, 137.0, 136.2, 135.0, 127.8, 127.4, 120.3, 74.4, 71.1, 58.9, 58.4, 54.0, 52.1, 50.3,40.8, 36.6, 35.8, 35.1, 33.9, 32.6, 30.4, 30.2, 29.3, 26.4, 24.9, 23.2, 23.0, 19.8, 19.2, 13.8, 4.0, 3.9; $[\alpha]^{25}_{365}$ = −199.9 (1.1 g/100 mL, 95/5 MeOH/water).

■ ASSOCIATED CONTENT

Supporting Information. HPLC analysis conditions for compounds **5**, **8**, **9**, **15**, **16**, **18**, **19**, **22**, **23**, and MK-7009; chiral assay methods for compounds **5**, **8**, and **15**; NMR spectra files for compounds **5-DCHA**, **8-PTSA**, **9**, **15**, **18-PTSA**, **19**, and MK-7009. This material is available free of charge via the Internet at http://pubs.acs.org/.

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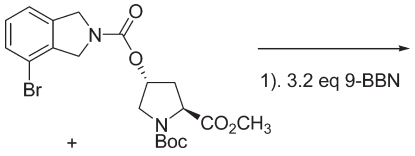
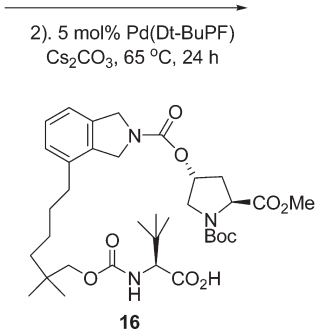


5-DCHA salt



16

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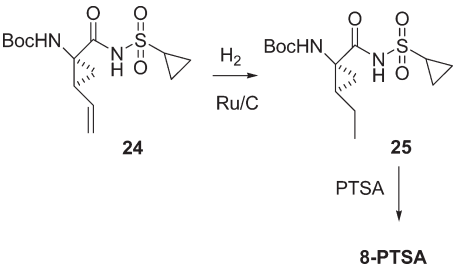
(44) In contrast to the ligandless Heck reaction, the Sonagashira reaction mixture could not be taken directly into the hydrogenation reaction as relatively poor conversions were obtained at high Pd/C loadings. We attribute this to catalyst poisoning by residual phosphine catalyst. As a result, aqueous workup followed by activated carbon treatment was required.

(45) The side chain was prepared from known compound **24** (ref 38) according to the scheme below.



24 **25** **8-PTSA**