

Total Synthesis of Enlicitide Decanoate

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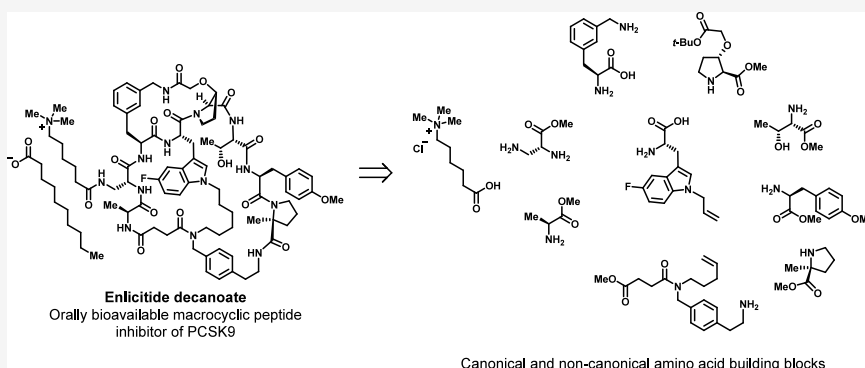
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ABSTRACT: We report the total synthesis of enlicitide decanoate, an orally bioavailable inhibitor of proprotein convertase subtilisin/kexin type 9 that is being developed for the treatment of atherosclerotic cardiovascular disease. It is a highly complex macrocyclic peptide with a significant number of nonpeptide structural elements that presents a daunting synthetic chemistry challenge. We describe the development of a convergent, efficient, and robust manufacturing process that enables the large-scale production of enlicitide.

INTRODUCTION

Enlicitide decanoate (**1**), also known as MK-0616, builds on a long and rich heritage of synthetic chemistry applied by the pharmaceutical industry to the discovery and development of highly complex small molecules for the treatment of human diseases (Figure 1). Two of the most well-known historical examples in this family are the antibiotic Vancocin (vancomycin, **2**)¹ and the chemotherapy agent Taxol (paclitaxel, **3**),² dazzling chemical structures that have attracted interest from total synthesis chemists for generations, though pharmaceutically relevant work has relied on semisynthetic methods from naturally derived precursors. The total synthesis of the anticancer drug Halaven (eribulin, **4**), an analog of the marine natural product halichondrin B, in over 60 steps by the chemistry team at Eisai stands as one of the most impressive feats of

polyketide manufacture.³ The Novartis chemistry team has published a 39 step synthesis of the tumor growth suppressing marine sponge isolate discodermolide, a tour de force based on synthetic strategies from the Smith and Paterson groups.⁴ Particularly in the field of oncology research, structurally complex and densely functionalized macrocyclic dinucleotides have become popular targets for industrial chemistry teams, as

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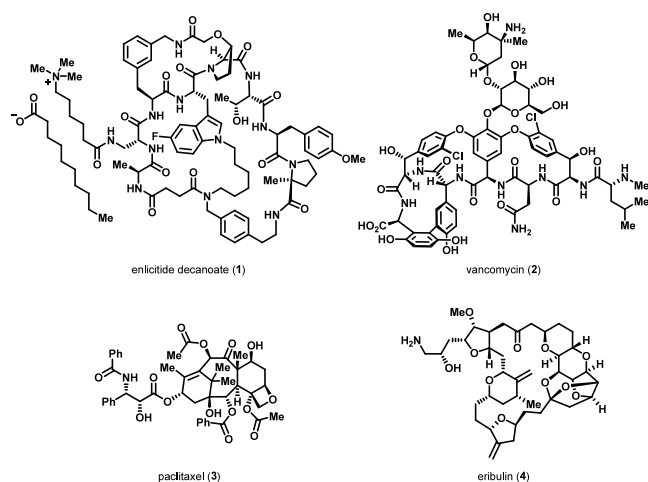


Figure 1. Enlicitide decanoate and other highly complex small molecules for the treatment of human diseases.

exemplified by seminal publications from Bristol Myers Squibb⁵ and us.⁶ A team at Amgen has recently disclosed a remarkable synthesis of the decorated macrocycle tapotoclax,⁷ which has been evaluated in clinical studies as an anticancer therapy.

From the perspective of a synthetic chemist, both the discovery and development of enlicitide clearly distinguishes it as a member of this molecular family. Except for tapotoclax, enlicitide is the only molecule whose structure was designed *de novo*,⁸ rather than existing as a natural product or close analog thereof. Additionally, enlicitide is being developed as a daily therapy for cholesterol management, for which worldwide demand may be substantial, not an oncology or novel antibiotic drug, for which clinical and commercial production volumes are generally modest. Enlicitide, an orally bioavailable inhibitor of proprotein convertase subtilisin/kexin type 9 (PCSK9) that has been shown in Phase 2 clinical studies to be well-tolerated and capable of delivering statistically significant and clinically meaningful reductions in low density lipoprotein cholesterol,⁹ is being developed for the treatment of atherosclerotic cardiovascular disease. Cardiovascular disease is the leading cause of death globally, having killed almost 19 million people in 2019 alone, according to the World Health Organization.¹⁰ Despite major reductions in mortality rates over the last century, it also remains the leading cause of death in the United States. Nearly 700,000 people died in 2021 from cardiovascular disease and many tens of millions more are at risk each year, according to the Centers for Disease Control and Prevention.¹¹ Substantial quantities of enlicitide are necessary to support its clinical development given the high number of patients required to demonstrate statistically significant reductions in the risk of myocardial infarction, stroke, and death, and far more will be needed to meet commercial global demand given the enormous disease burden. It has been well-recognized that molecular complexity drives the necessity for continued innovation in the process development sciences,¹² and the molecular complexity and size of enlicitide – paired with the need to prepare hundreds of kilograms to support its clinical evaluation – placed substantial constraints on any chemical synthesis we designed. Enlicitide had to be built in a way that not only optimized cost and speed, but that enabled very large-scale production and significant material throughput. The synthesis should be as convergent as possible to shorten the overall production time cycle and it would have to minimize chromatographic

purifications (which are costly, time-consuming, and inefficient) throughout the route, if not avoid them altogether.

While enlicitide was developed from an mRNA display screen of highly peptidic monocyclic macrocycles, it contains significant nonpeptidic structural elements. These include several macrocyclic cross-links that severely limit the utility of solid phase peptide synthesis, the standard technology widely employed to manufacture synthetic peptides. The molecule is comprised of eight amino acids, two of which are canonical (9 and 11) and six of which are noncanonical (5, 6, 7, 8, 12, and 14) (Figure 2). In addition, the 37-membered and largest macro-

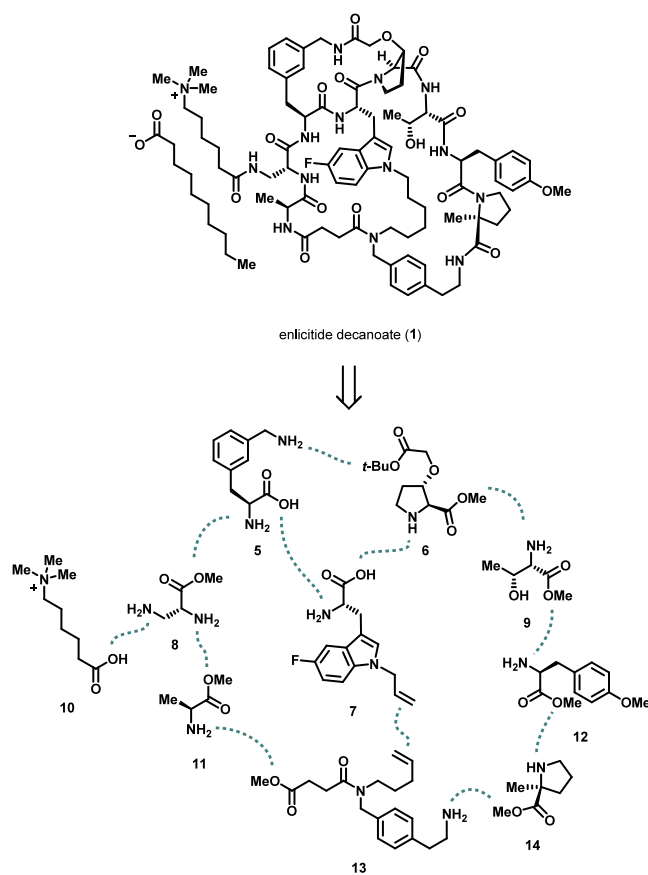
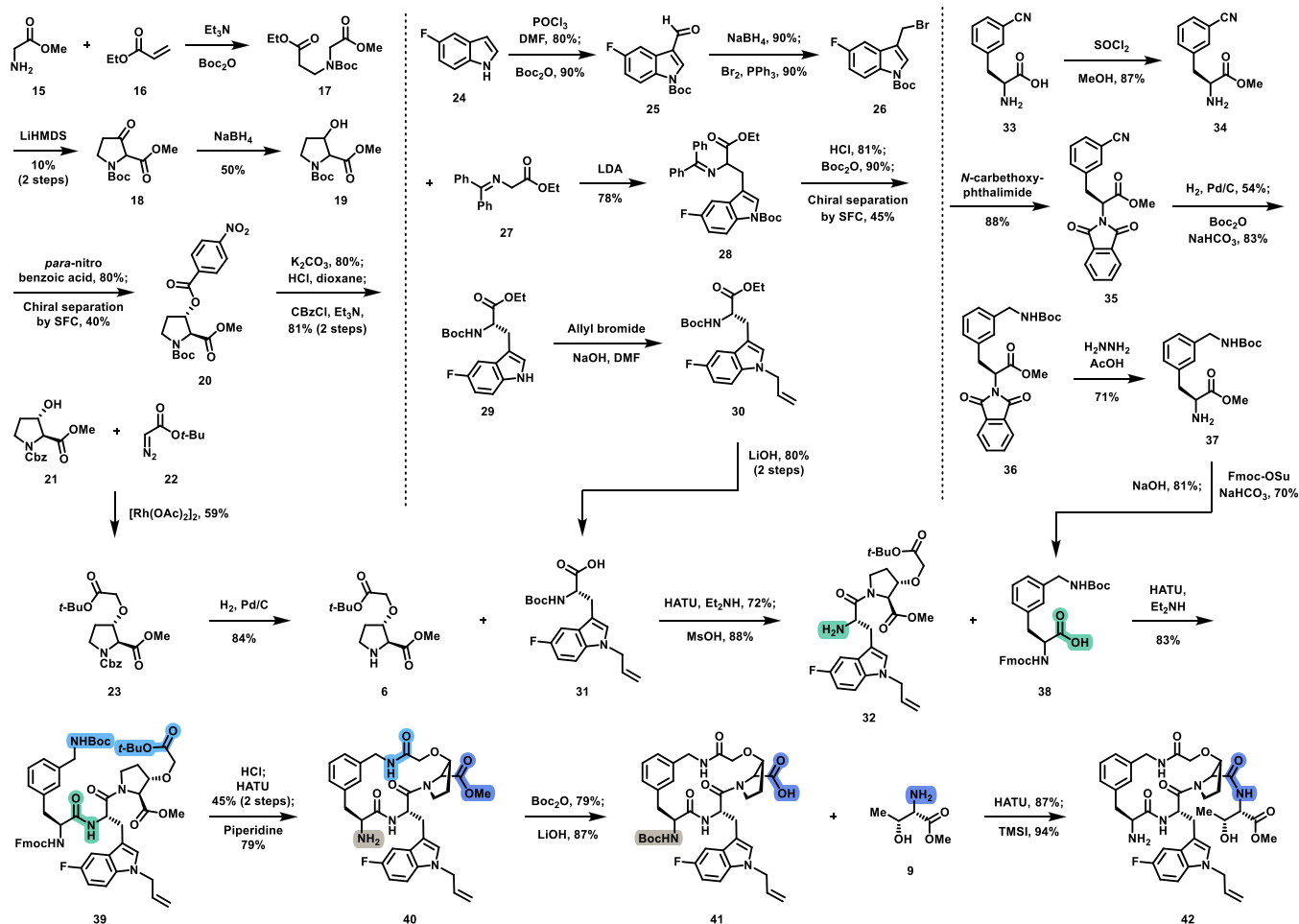


Figure 2. Enlicitide decanoate and its building blocks.

cycle contains an elaborated nonpeptidic fragment (13) and is appended with an ammonium-containing side chain (10). In recognition that exhaustive experimentation to establish the best order of assembly for these ten building blocks, in addition to the most efficient bonds to target for the requisite macrocyclizations, was simply impractical, several initial strategic synthetic choices were made to focus our efforts. We have previously reported early synthetic forays into molecules of this general class.¹³ One pivotal choice was to first target the assembly of what would come to be known as the Northern fragment, the 17-membered macrocycle formed by the coupling of noncanonical amino acids 5, 6, and 7. With that toehold, prioritization was given to routes that offered the greatest simplification in a retrosynthetic sense, including disconnecting of the larger macrocycle of the Northern fragment itself (e.g., between amino acids 5 and 8); more discussion of this synthetic logic is provided later in this report. Anticipating significant challenges associated with carrying any highly polar, water-soluble ammonium-containing intermediate through multiple synthetic steps and

Scheme 1. First Generation Synthesis of the Enlicitide Northern Fragment



the attendant need to carefully control its counterion at every unit operation, we decided to introduce the side chain (10) at the very end of the synthesis. Another early decision was to rely on a ring-closing metathesis reaction to form the *n*-hexyl link between 7 and 13, with the confidence that such a generally orthogonal transformation could be efficiently performed at multiple points in the overall process, enabling flexibility with respect to other aspects of the synthesis as greater understanding of the available route options was gained. With the strategy provided by this integrated set of choices, we embarked on an unparalleled journey to establish a synthetic route to enlicitide and develop a robust, highly scalable process to enable the clinical evaluation of its safety and efficacy.

RESULTS AND DISCUSSION

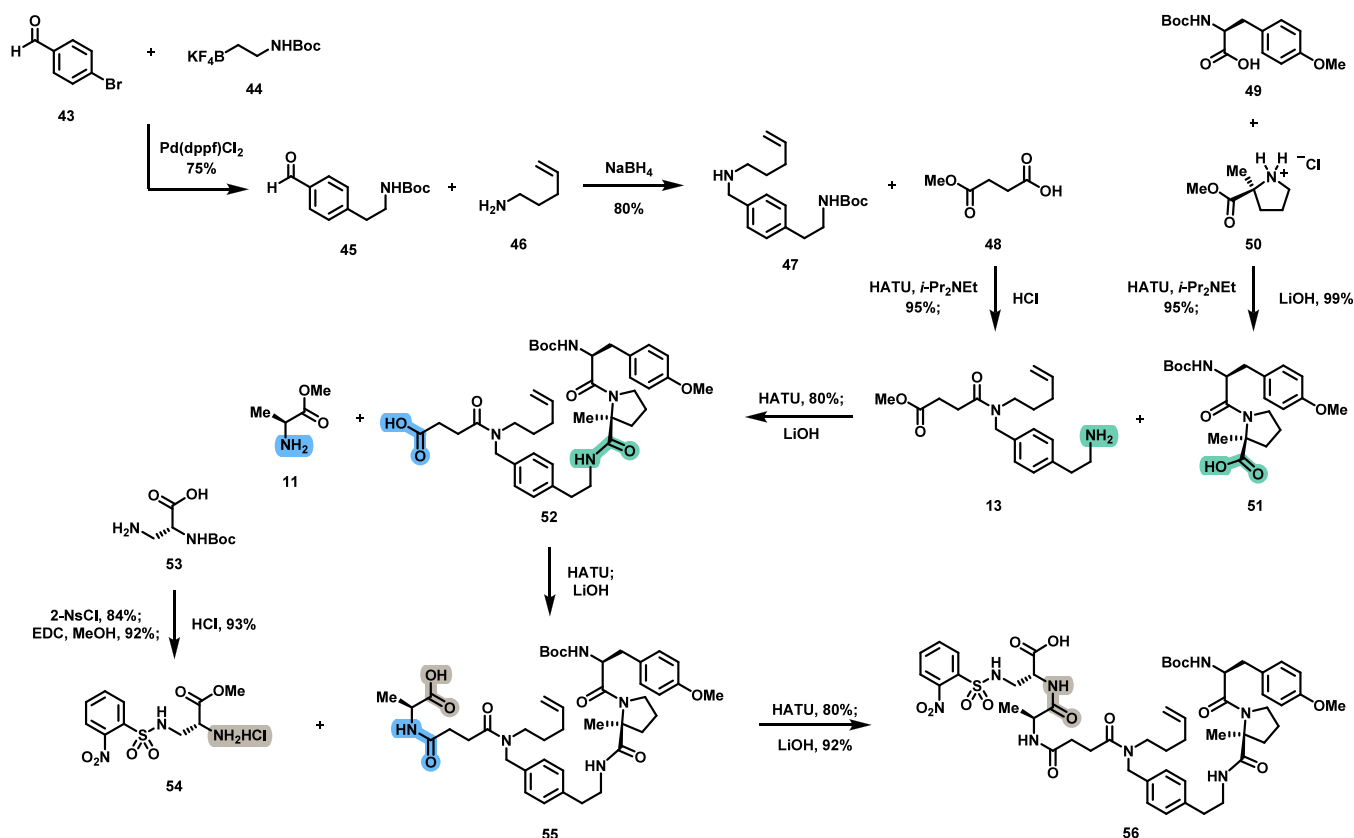
First Generation Synthesis of Enlicitide. Our synthesis of the first generation enlicitide Northern fragment (42) began with the preparation of its three constituent noncanonical amino acids (6, 31, and 38, Scheme 1). Each were synthesized with orthogonal protecting groups, both to provide flexibility for multiple synthetic options to bring together the Northern fragment and to provide material that would be as broadly useful as possible for ongoing medicinal chemistry efforts to identify new PCSK9 inhibitors. The synthesis of the elaborated 3-hydroxyproline-based amino acid (6) began with the coupling of methyl glycinate (15) and ethyl acrylate (16), followed by protection of the resulting secondary amine to give diester 17.

Dieckmann condensation gave the corresponding cyclic β -keto ester (18), though oligomerization was competitive with the desired intramolecular cyclization, which lowered the efficiency of both the reaction and the product isolation. Nevertheless, a useful quantity of the desired material could be obtained, which was reduced diastereoselectively to the β -alkoxy ester (19). Acylation with para-nitrobenzoic acid enabled preparative separation and isolation via chiral supercritical fluid chromatography (SFC) of the desired enantiomer (20). Deprotection of the alcohol and an exchange of protecting groups on the ester and amine functionalities was followed by a dirhodium(II) tetraacetate-catalyzed O–H insertion of *tert*-butyl diazoacetate (22). Finally, hydrogenolysis of the nitrogen protecting group with heterogeneous palladium catalysis provided the non-canonical amino acid (6).

The synthesis of the *N*-allyl-5-fluorotryptophan-based amino acid (31) began with a direct formylation of 5-fluoroindole (24). Protection of the indole nitrogen and conversion of the resulting aldehyde (25) to the corresponding bromomethylindole (26) allowed for coupling with the benzophenone imine of glycine ethyl ester (27). After a protecting group exchange, chiral SFC purification yielded the desired enantiomer (29). Addition of allyl bromide to the indole and saponification of the ethyl ester gave noncanonical amino acid 31.

The aminomethyl-substituted phenylalanine 38 could be readily accessed from the structurally analogous and commercially available nitrile-containing noncanonical amino acid (33).

Scheme 2. First Generation Synthesis of the Enlicitide Southern Fragment



Conversion of that material to the corresponding protected methyl ester (35) enabled selective hydrogenation of the aryl nitrile group under heterogeneous palladium catalysis. Protecting and functional group manipulations were then performed to deliver the desired orthogonally protected building block (38).

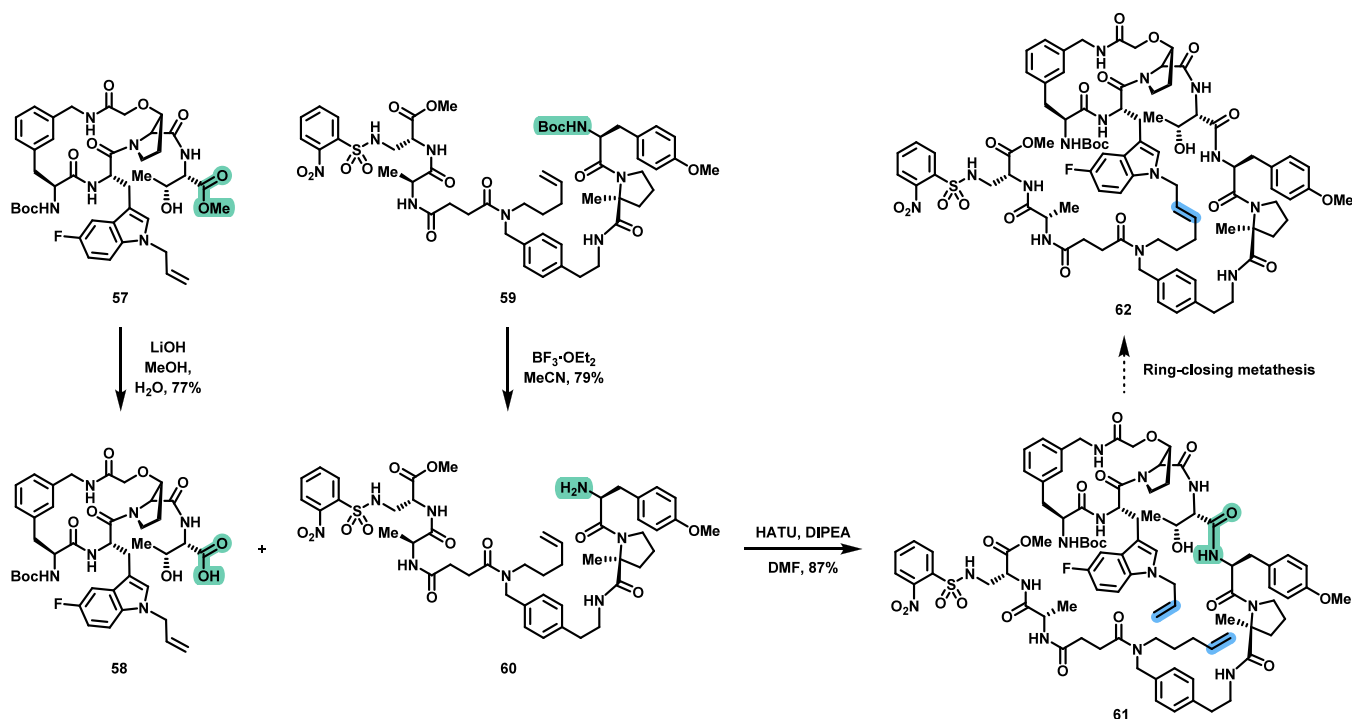
Forming the three requisite amide bonds of the first generation enlicitide Northern fragment (42) between the three noncanonical amino acids proceeded smoothly, first through the union of 6 and 31 and partial deprotection to dipeptide 32, followed by the addition of 38. The tripeptide (39) could then be selectively deprotected to set the stage for the macrolactamization. While high dilution was required to avoid significant oligomerization during the cyclization, sufficient quantities of the desired macrocycle (40) could be obtained. Finally, addition of L-threonine methyl ester (9) to the C-terminus of the macrocyclic peptide completed the synthesis of 42. This sequence consisted of 37 total steps from starting materials available on large scale from commercial suppliers, 20 steps of which were in the longest linear sequence.

Synthesis of the first generation enlicitide Southern fragment (56) also began with preparing orthogonally protected building blocks to enable both high selectivity and flexibility during the fragment assembly stage (Scheme 2). Synthesis of the nonpeptidic portion of the Southern fragment started with a Suzuki cross-coupling of 4-bromobenzaldehyde (43) and a suitable alkyl tetrafluoroborate (44) to yield the elaborated benzaldehyde 45. Reductive amination with pent-4-en-1-amine (46) to 47 and amidation with 4-methoxy-4-oxobutanoic acid (48) provided intermediate 13. This could be subsequently coupled with dipeptide 51, derived from an amidation reaction followed by saponification between Boc-O-methyl-L-tyrosine (49) and the hydrochloride salt of 2-methyl-L-proline (50).

Commercially available monoprotected 3-amino-D-alanine (53) was further protected with 2-nitrobenzenesulfonyl chloride and esterified to 54. A nosyl protecting group was chosen for this primary amine based on our strategic choice to introduce the side chain of enlicitide as the last step. We believed that a nosyl group would be robust enough to carry through all the preceding transformations, enabling this particular protected amine to be chemoselectively revealed only at the penultimate step in the synthesis. Iterative selective deprotections and peptide couplings, first between L-alanine methyl ester (11) and 52, and then with noncanonical amino acid 54, completed the first generation enlicitide Southern fragment (56). This sequence consisted of 15 total steps from starting materials available on large scale from commercial suppliers, 9 steps of which were in the longest linear sequence.

Stitching together the Northern and Southern fragments of enlicitide required connections between three embedded building blocks, to wit: the amidation between 5 and 8, a ring-closing or cross-metathesis between 7 and 13 (depending on whether the first fragment coupling step was amidation or metathesis), and the amidation between 9 and 12 (Figure 2). Time constraints did not permit an exhaustive evaluation of all six possible ways to sequence these three transformations (in addition to the necessary work to evaluate the intermediate protecting group manipulations), but our previous work on molecules of this type provided confidence that the final cyclization to form the largest macrocycle, either between amino acids 5 and 8 or 9 and 12, would be a viable synthetic pathway to complete the core structure. With relatively unbiased terminal alkenes in both fragments, it seemed unlikely that a selective cross-metathesis would prove to be an efficient method for the initial fragment coupling step, so the first choice was to bring

Scheme 3. Attempted Ring-Closing Metathesis Route toward Enlicitide



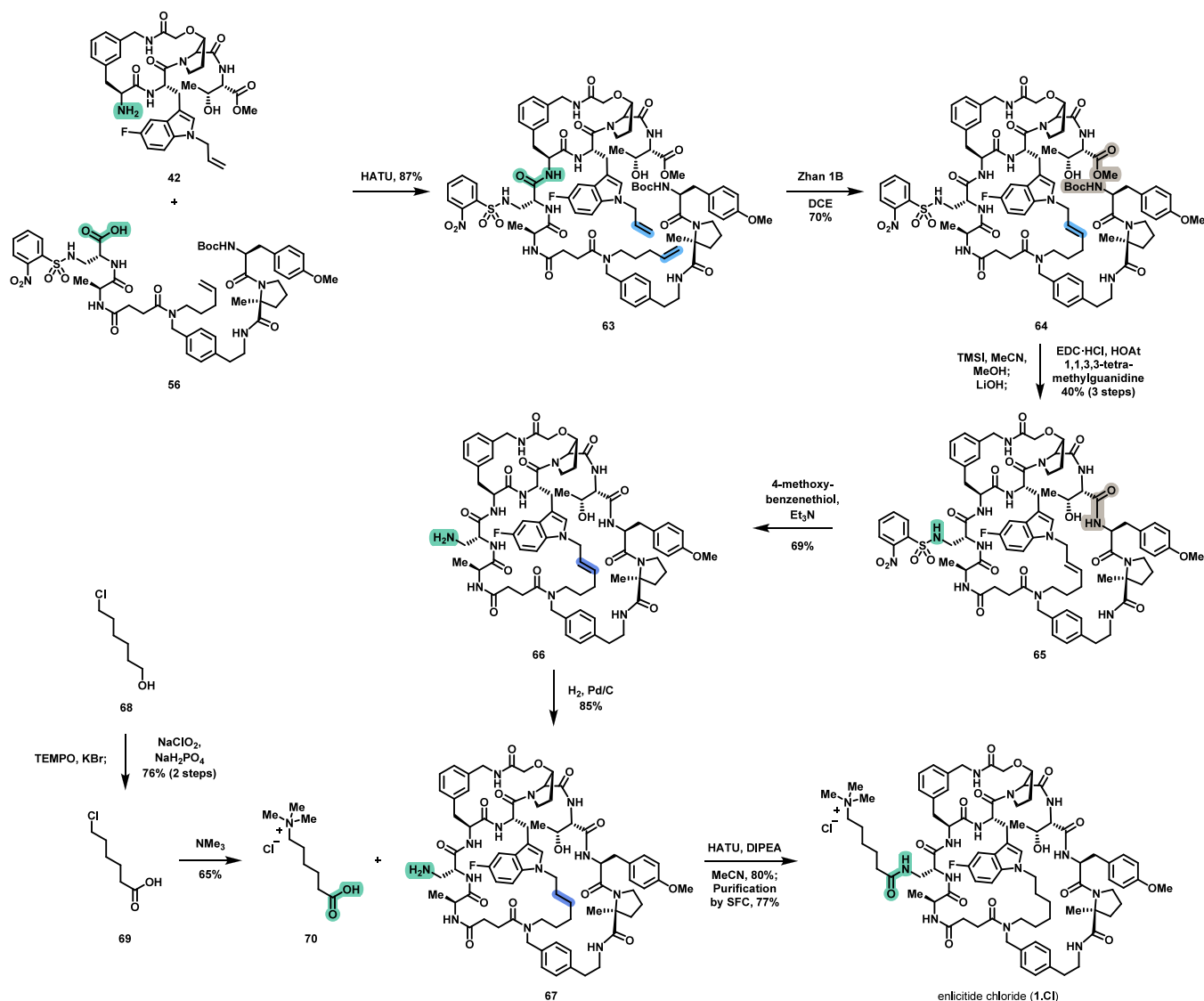
together the Northern and Southern fragments with one of the two possible amidations. A persistent challenge from this point of the synthesis onward was the exceedingly poor physical properties of every late stage intermediate. Most were poorly soluble in nearly all solvents, including water, significantly complicating the reaction, workup, and isolation phases of each process. No aqueous workups could be practically employed once the Northern and Southern fragments were connected, which required substantial efforts to identify suitable conditions for amorphous precipitation at the end of each reaction to aid both isolation and, to at least some modest extent, purification before proceeding to the next step. With so many potentially reactive functional groups at this stage, and the increasing risk of epimerization under acidic or basic conditions – compounded by the increasing likelihood that any epimerization would prove challenging to detect as the structural complexity grew – priority was given to the mildest conditions possible at each stage.

We evaluated the viability of an end game strategy starting with the coupling between building blocks 9 and 12 (Figure 2). The penultimate intermediates for both the first generation Northern and Southern fragments could be readily deprotected to enable an assessment of the first amidation. After saponification of 57 and deprotection of 59, we were able to efficiently perform a HATU-mediated amidation to give the linear precursor 61 (Scheme 3). Unfortunately, despite extensive catalyst and reaction parameter screening, only small amounts of the desired ring-closed product (62) could be detected. Given the later success of a ring-closing metathesis reaction between these alkenes on a different precursor, we hypothesize that under the conditions evaluated, the solution state conformation of the linear precursor was unfavorable for the desired transformation. With only trace quantities of the ring-closed product, we were unable to evaluate the subsequent macrolactamization to complete the core of enlicitide through this route.

Fortunately, an alternative sequence of these steps proved substantially more successful and enabled the completion of the first scalable synthesis of MK-0616 (Scheme 4). As before, a HATU-mediated coupling was performed to couple the Northern (42) and Southern (56) fragments, but this time between amino acids 6 and 8 instead of 9 and 12 (Figure 2). Gratifying, this linear precursor (63) was far less recalcitrant to ring-closing metathesis. While high dilution was required to minimize oligomer formation through unproductive cross-metathesis reactions, the desired macrocycle (64) could be obtained in 70% isolated yield after chromatographic purification. Sequential saponification and amine deprotection set the stage for the macrolactamization, which could be smoothly performed to afford sufficient quantities of the macrocycle (65). Benzenethiol, a standard reagent for nosyl group removal, proved insufficiently reactive in this complex system. However, its more nucleophilic 4-methoxy analog was able to perform the deprotection while maintaining the desired chemoselectivity. Heterogeneous palladium-catalyzed hydrogenation of the resultant alkene from the ring-closing metathesis completed the *n*-hexyl linker, and finally deprotection of the nosyl group completed the core (67) of enlicitide. The ammonium-containing side chain (70) was derived from 6-chlorohexan-1-ol (68), which was converted to the corresponding carboxylic acid through sequential oxidations; subsequent displacement of the alkyl chloride with trimethylamine completed the side chain. Finally, a high-yielding amidation to install the side chain onto the core of the molecule followed by SFC purification completed the first generation synthesis of enlicitide as its chloride salt (1.Cl) in 63 total steps, 28 of which were in the longest linear sequence.

Further Development of the Northern Fragment Synthesis. While this total synthesis was able to be supply initial quantities of material for early studies, it was not nearly efficient enough for the development of enlicitide. The practical constraints of having to perform so many synthetic steps and all

Scheme 4. First Generation End Game Synthesis of Enlicitide



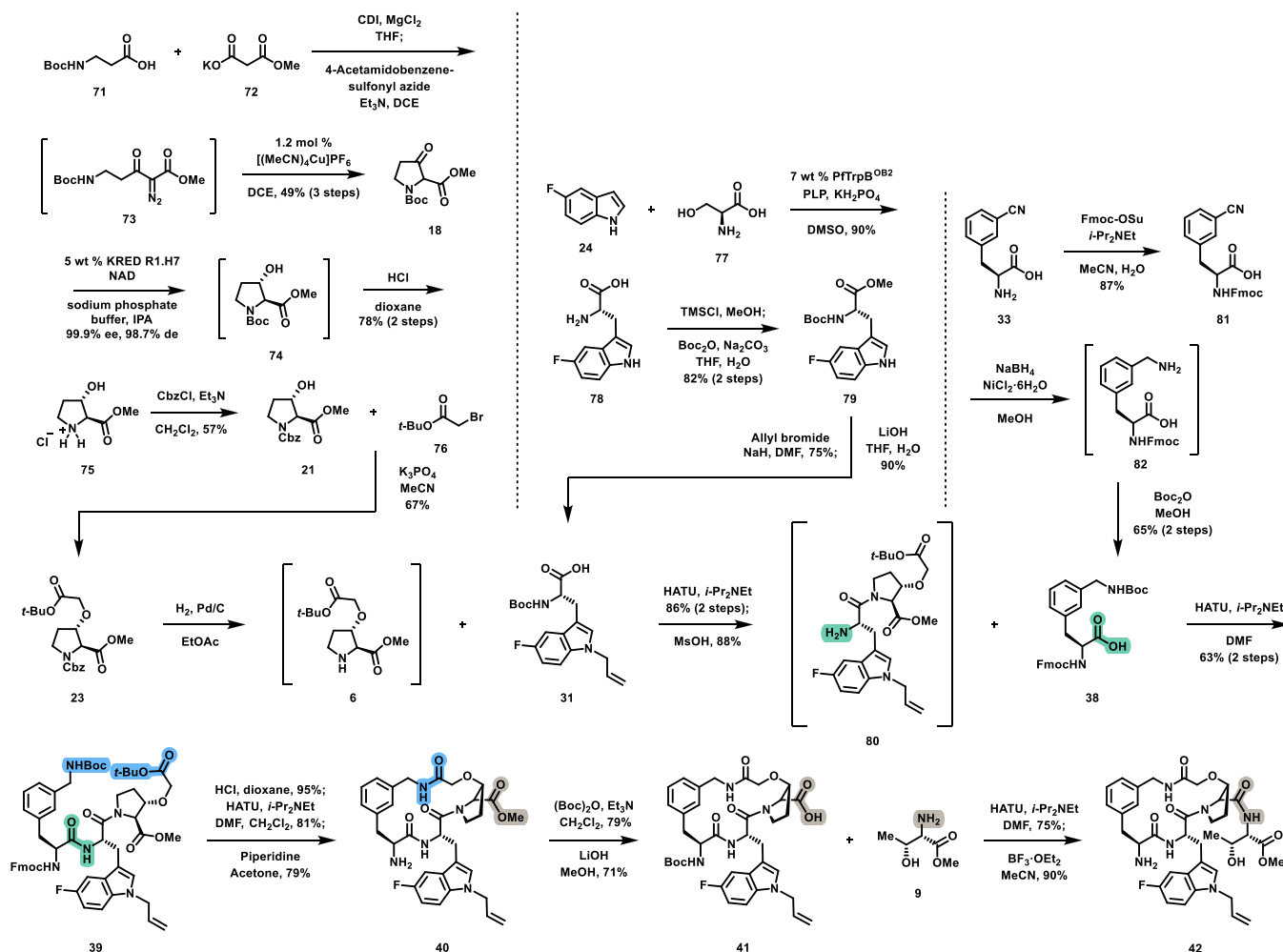
the associated unit operations, on top of several laborious chromatographic purifications, would make any large-scale production challenging and hence slow serious preclinical or clinical evaluation. Thus, before we had even finished the first generation synthesis of enlicitide, work began in earnest to applying our new insights into the construction of this molecule to develop a significantly better route.

Our attention first turned to the Northern fragment, the synthesis of which contained over half the total steps. Though we had shown that the hydroxyproline-containing noncanonical amino acid **6** was a suitable building block en route to the fragment, *5-exo-trig* cyclization to form the necessary pyrrolidine ring was quite poor and neither of the two stereocenters were subsequently set selectively. In our second generation synthesis, we elected to pursue a mechanistically different cyclization method, relying instead on a carbene-mediated N–H insertion (Scheme 5). To begin, Claisen condensation of *N*-Boc-3-(methylamino)propanoic acid (**71**) and potassium methyl malonate (**72**), followed by base-induced diazo transfer to form **73**, set the stage for a decomposition to a copper-carbene that efficiently underwent the desired N–H insertion to provide the corresponding cyclic β -keto ester (**18**). Ketoreductases are

well-known to selectively reduce ketones to alcohols, and we were gratified to identify in a high throughput screen an available enzyme that was able to convert the cyclic β -keto ester to the corresponding β -hydroxy ester (**74**) and set two stereocenters with high chemo- and stereoselectivity;¹⁴ removal of the Boc protecting group was performed *in situ* to give the corresponding hydrochloride salt (**75**). Instead of utilizing a rhodium-catalyzed O–H insertion to append the *tert*-butyl acetate group to the alcohol as in the first generation synthesis, we instead performed an alkylation with *tert*-butyl 2-bromoacetate (**76**). As before, palladium-catalyzed heterogeneous hydrogenation provided the noncanonical amino acid (**6**).

Like our first generation synthesis of **6**, the initial route to the tryptophan-containing noncanonical amino acid (**31**) was nonstereoselective and required an inefficient chiral separation to provide a single enantiomer of the building block. In 2015, the Arnold group published seminal work on the application of protein engineering to create highly efficient variants of the tryptophan synthase β -subunit (TrpB) that can be highly efficient for the production of tryptophan-like noncanonical amino acids.¹⁵ We were able to apply this technology to our synthesis, using a custom TrpB variant to unite 5-fluoroindole

Scheme 5. Second Generation Synthesis of the Enlicitide Northern Fragment



(24) and L-serine (77) in high yield and stereoselectivity to noncanonical amino acid 78. Esterification and protecting group manipulations enabled, as before, installation of the allyl group on the indole nitrogen to complete the building block (31).

Optimization of the synthetic sequence, including the protecting group strategy, of the aminomethyl-substituted phenylalanine (**38**) provided meaningful improvements in the material throughput to the remaining noncanonical amino acid. With the three building blocks in hand, assembly to the Northern fragment proceeded in line with the first generation synthesis, with significant efficiencies gained by being able to carry several intermediates forward directly, avoiding several challenging isolations. The second generation synthesis required 26 total steps, 11 fewer than the 37 of the first, and reduced the longest linear sequence from 20 to 18 steps.

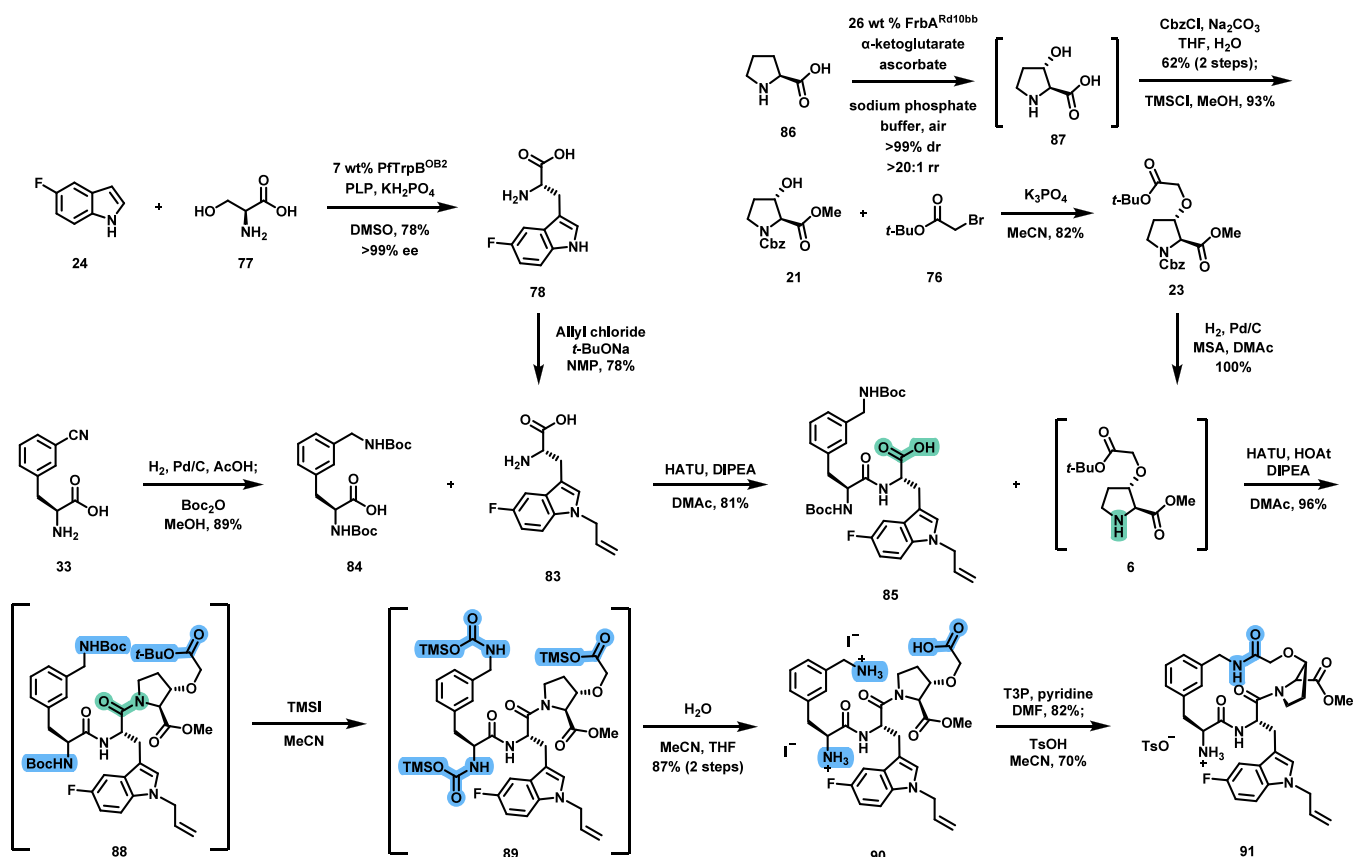
This much-improved route to the enlicitide Northern fragment was used for a time to enable the program to begin clinical development, but several strategic opportunities created the need for an even further optimized route to the fragment. The first was the pivotal discovery of three late-stage crystalline intermediates, which provided such important points of purity control that diverting the route to intercept all three was of significant benefit. The second was a desire to conduct the ring-closing metathesis as late as possible in the synthesis. Given the ruthenium catalyst's high contribution to the overall cost of the

manufacture of enlicitide, performing this reaction near the end of the route provided significant cost savings.

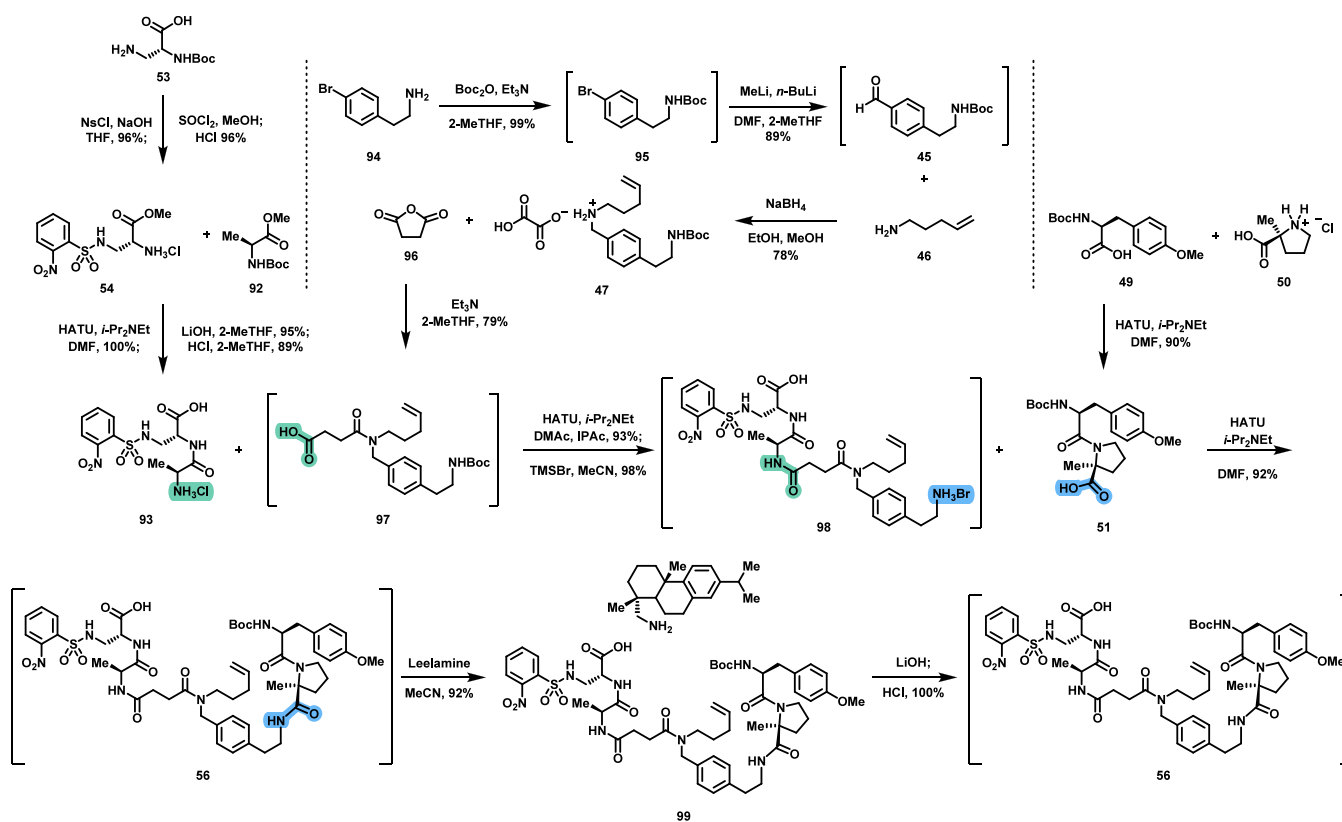
The third generation synthesis of the enlicitide Northern fragment targeted a different version of this fragment than was completed in either of the first two generations. Lacking the L-threonine amino acid, which would be installed later, this choice was driven by the identification of crystalline tosylate salt **91** (Scheme 6), which could be isolated in good yield and purity and provided a highly stable intermediate that could be stockpiled as a key intermediate during the synthesis.

Despite the substantial improvements that had already been realized through the application of both the copper-catalyzed N–H insertion to construct pyrrolidine **18** and the KRED dynamic kinetic diastereoselective reduction to form the β -hydroxy ester (Scheme 5), we continued to explore even more productive syntheses to the hydroxyproline-containing non-canonical amino acid. Ultimately the most efficient route centered around an α -ketoglutarate-dependent oxidation of L-proline (**86**) with a proline hydroxylase variant to directly install the desired hydroxyl group, which proceeded in >99:1 diastereoselectivity at the 3-position of the proline ring and favoring by >20:1 3-hydroxylation over 4-hydroxylation (Scheme 6).¹⁶ Masking of the nitrogen with a CBz protecting group and esterification enabled isolation of the intermediate (**21**) from the aqueous system needed for the oxidation step, which was followed by alkylation to install the *tert*-butyl acetate

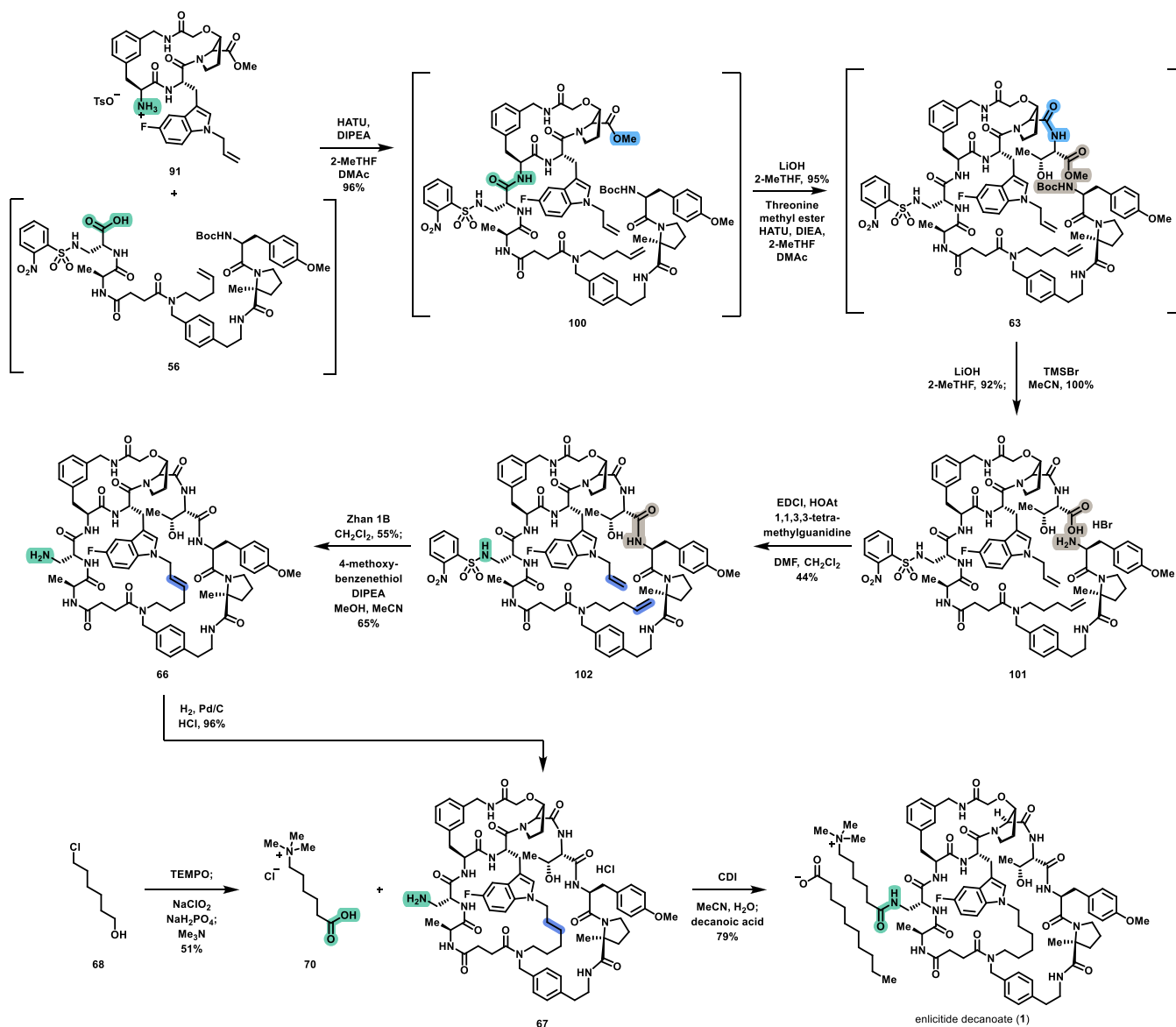
Scheme 6. Third Generation Synthesis of the Enlicitide Northern Fragment



Scheme 7. Second Generation Synthesis of the Enlicitide Southern Fragment



Scheme 8. Second Generation End Game Synthesis of Enlicitide



group and deprotection to **6** as per the second generation synthesis.

Development of a direct allylation of the TrpB product (**78**) avoided the need for some of the protecting group chemistry that was necessary in the previous synthesis, and reordering the coupling of the three building blocks avoided the need to esterify the tryptophan acid. This route change also simplified the protecting group needs of the aminomethyl-substituted phenylalanine (**84**), allowing Boc to be employed for both nitrogen functional groups, shortening the route to only two steps.

By first activating the carboxylic acid of **84** with HATU before adding **83**, the desired amidation could be performed without the need to protect the carboxyl acid of **83**. As before, we were able to utilize the crude reaction stream from the hydrogenolysis of **23** to perform the second coupling to form tripeptide **88**. The previous two syntheses relied on selective deprotection of the less hindered nitrogen of the aminomethyl-substituted phenylalanine to selectively form the desired macrocycle of the Northern fragment, but we hypothesized that the different steric environments and pK_a of each nitrogen would be sufficiently

differentiating during the cyclization. We were gratified to discover that in the preparation of the necessary substrate to assess this idea, we discovered a crystalline salt of the uncyclized intermediate (**90**), which provided yet another opportunity for purity upgrade. Free-basing this salt and then amidation with T3P proceeded smoothly; addition of TsOH enabled the isolation of the newly designed Northern fragment (**91**) in only 14 total steps, only about a third as many steps as were needed in the first generation synthesis. Just as importantly, the longest linear sequence had been more than halved over the course of the three generations, from 20 steps to merely 9.

Further Development of the Southern Fragment Synthesis. The improvement realized in the second generation synthesis of the enlicitide Southern fragment was not to the step count (total or longest linear), but the overall efficiency and purity control (Scheme 7). Since the first synthesis of enlicitide, we had been investing in expansive high-throughput screening campaigns in the hopes of identifying crystalline intermediates. Part of this work was to screen large libraries of potential salt pairs or cofomers that might induce crystallinity when efforts to

crystallize a free base had failed. Out of thousands of experiments, a single hit was identified: a leelamine salt of the Southern fragment (**99**). The successful use of this diterpene, which has previously been shown to have utility as a chiral resolving agent for carboxylic acids,¹⁷ in forming a crystalline salt (**99**) was highly unexpected yet could be relied upon to robustly isolate pure material, avoiding the need for the extensive normal phase chromatography that the previous generation synthesis required. Formation and then breaking of the leelamine salt added addition steps to the fragment synthesis, but this price was more than offset by the benefits a late-stage crystalline intermediate gave us.

The new route to the Southern fragment relied as before on monoprotected 3-amino-D-alanine (**53**), Boc-O-methyl-L-tyrosine (**49**), and the hydrochloride salt of 2-methyl-L-proline (**50**). The synthesis of the nonpeptidic portion of the Southern fragment was refined given the new target of the free carboxylic acid. Boc protection of (4-bromophenyl)ethan-1-amine (**94**), followed by lithium-halogen exchange and formylation with DMF gave the corresponding benzaldehyde (**45**), which was carried forward without isolation to a reduction amination with pent-4-en-1-amine (**46**). The resulting secondary amine could readily react with succinic anhydride to provide the corresponding ring-opened acid (**97**), which could be coupled with both dipeptide pieces of the Southern fragment to set the stage for the leelamine-enabled crystallization. Finally, free base of the salt (**99**) and formation of the protonated acid (**56**) completed the second generation synthesis of the Southern fragment in 16 total steps, 11 in the longest linear sequence.

Completion of the Second Generation Synthesis of Enlicitide. With optimized routes to the Northern and Southern fragments of enlicitide, both now able to take advantage of late-stage crystalline intermediates, we were able to advance to the end game of the synthesis (Scheme 8). Once again, the strategy unfolded from an intermolecular amidation between amino acids **5** and **8** (Figure 2). This coupling was sufficiently clean to enable the crude reaction stream to be carried forward several steps before the next isolation point (**101**). First, saponification of **100** revealed the carboxylic acid of the Northern fragment C-terminus, to which could now be coupled L-threonine. A second saponification, this time of the L-threonine methyl ester (**63**), and removal of the Boc protecting group of L-tyrosine, gave the full linear precursor to the enlicitide core as the amino acid (**101**). Again, with the desire to perform the ring-closing metathesis as close to the finish as possible, it was this intermediate at which we opted to perform the macrolactamization to **102**. The subsequent trans-annular ring-closing metathesis completed the formation of the enlicitide core, with only the hydrogenation of the vestigial alkene and removal of the nosyl protecting group required. Fortunately, our broad crystallization experiments paid off again late in the synthesis. Crystalline forms were discovered for the two intermediates before and after the RCM reaction, **102** and **66**, providing opportunities for optimization around this key step. Extensive process optimization followed, including the development of a multicomponent and multistage liquid–liquid extraction process leveraging the solubility and pK_a differences, ultimately resulting in a robust and scalable isolation of **66** that improved the overall purity significantly by both removing unrelated compounds and several high molecular weight oligomers and epimers generated by the upstream process. Surprisingly, the analogous alkane (**67**), which differs by only two hydrogen atoms in a compound with a molecular weight of

nearly 1400, proved much more challenging to crystallize under any conditions we evaluated. Our hypothesis is that despite the seemingly small structural change imparted by the hydrogenation of the internal alkene, significant solution state structure changes must be at play, inhibiting crystallization of **67**, and conversely enabling the crystallization of **66**. We continued to utilize the synthesis of the ammonium-containing side chain developed during the first generation efforts, and the amidation to couple it to the core of the molecule proceeded without event. One of the most significant achievements of this optimized route was the discovery and development of a crystallization process to isolate and purify enlicitide, now as its decanoate instead of chloride salt, obviating the need for the time-consuming, wasteful, and expensive SFC process that the prior synthesis had relied upon. Large scale chromatography was in fact so inefficient for this molecule that this innovation alone reduced both the total production time cycle and cost by a third. Overall, the second generation synthesis of enlicitide required 43 steps, 21 steps of which were in the longest linear sequence. This sequence is a third less in both total step count and steps in the longest linear sequence of our first generation process.

CONCLUSION

With this second generation process, despite the daunting challenge posed to us by enlicitide, we achieved our objectives of developing synthetic chemistry that enables large-scale production and optimizes efficiency, cost, and speed. As one measure of both the high productivity of this improved route and the extraordinary improvement it represents over the original synthesis, it is illustrative to consider the requirements of the earliest Northern fragment raw materials, which begin the longest linear sequence to enlicitide. The first generation process begins with methyl glycinate (**15**) and ethyl acrylate (**16**), which are both readily available on commercial scale. Yet with the overall yield between them and enlicitide of approximately 0.00007%, to manufacture even a single 100 kg batch of enlicitide would necessitate the procurement and processing of over 170 MT of these two commodities through nearly 30 synthetic steps. The second generation process, for which the analogous longest linear sequence through the Northern fragment begins with L-proline (**86**), proceeds in nearly 1000 times greater overall yield and thus requires only about 100 kg of this amino acid to make 100 kg of enlicitide. We look forward to applying the strategies and technologies described herein, developed over many years by a large and diverse team of fearless scientists, to a new generation of such macrocyclic peptides that hold the promise of differentiated abilities to save and improve lives of patients, worldwide.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.4c15966>.

Experimental procedures and characterization data for the second generation process to manufacture enlicitide (PDF)

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Notes

The authors declare no competing financial interest.

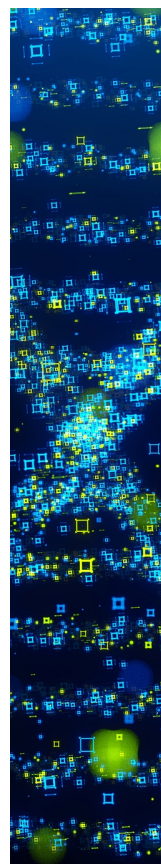
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