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A Divergent Route to Eravacycline

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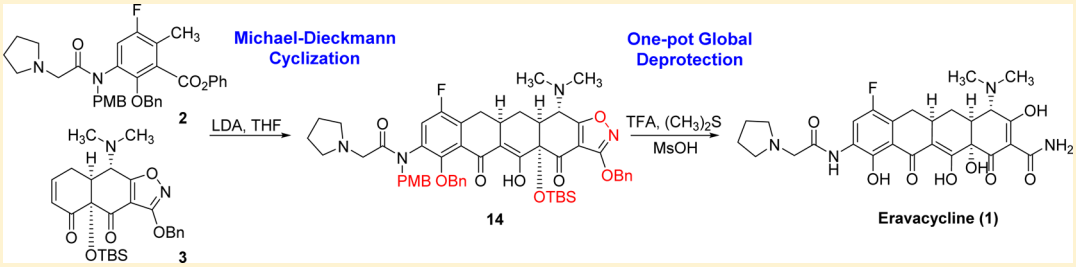
Supporting Information



ABSTRACT: A convergent route to eravacycline (**1**) has been developed by employing Michael−Dieckmann cyclization between enone **3** and a fully built and protected left-hand piece (LHP, **2**). After construction of the core eravacycline structure, a deprotection reaction was developed, allowing for the isoxazole ring opening and global deprotection to be achieved in one pot. The LHP is synthesized from readily available 4-fluoro-3-methylphenol in six steps featuring a palladium-catalyzed phenyl carboxylation in the last step.

INTRODUCTION

Eravacycline is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class being developed for the treatment of serious infections, including those caused by multidrug-resistant pathogens. To date, eravacycline has been tested in two phase 3 clinical trials.[1] As part of our product development activities, we needed to prepare ^{14}C-labeled eravacycline. While ^{14}C-eravacycline can be easily accessed by appending a labeled acetamide moiety to the C9 position of the core, it is often preferable to install the ^{14}C atom within the core (i.e., the ring system) of the molecule to avoid loss of radioactivity via biotransformation. The preparation of such a ^{14}C-labeled analogue brings unique synthetic challenges. In most cases, the practical synthesis of ^{14}C-labeled material requires (i) introduction of the ^{14}C moiety as late as possible in order to minimize the number of "hot" steps given the valuable ^{14}C starting material and the high cost of licensed radioactive waste disposal and (ii) the use of widely available ^{14}C-labeled starting materials, which are normally prepared from ^{14}C barium carbonate.[2] The current eravacycline manufacturing process,[3] which uses two key building blocks[4] and introduces the side chain in the last step, does not appear to be suitable to accomplish these goals. We therefore decided to embark on a new synthetic route to eravacycline in which the 11-position carbon, a desired spot for radiolabeling, is introduced late in the synthetic sequence. In the present paper, this new route is carried out with nonradioactive material and could eventually enable the practical synthesis of a ^{14}C-labeled analogue with high specific activity.

We planned to construct the eravacycline core structure via a Michael−Dieckmann reaction of enone **2**[4a,b] and a fully built left-hand piece (LHP, **3**), a general strategy for tetracycline synthesis developed by Myers.[5] The ^{14}C can be introduced in the last step of LHP synthesis and installed at the 11-position of eravacycline (Scheme 1). With this route, we envisioned that only three to five steps would need to be performed in a radiolabeling laboratory. This strategy should be applicable to other tetracycline analogues currently under development at Tetraphase.

RESULTS AND DISCUSSION

Synthesis of Bromide 4. We designed a five-step concise route to synthesize the highly substituted aryl bromide **4** as depicted in Scheme 2 starting from commercially available 4-fluoro-3-methylphenol (**5**). Dibromination of **5** using molecule bromine can be easily accomplished in DCM or in acetic acid. However, the product **6** is intrinsically not stable, and homodimerization occurs during the reaction, especially during the basic workup and concentration. Up to 20% of dimerization product could be present in the bromination product. We found bromination and subsequent Zincke nitration[6] could be performed in one pot using acetic acid as the common solvent. Homodimerization was minimized by careful addition of $NaNO_2$ into the bromination reaction mixture. Nitro displacement selectively occurred at the less hindered 6-position to give nitrophenol **7**, which was purified by recrystallization from aqueous acetonitrile. Subsequent benzylation afforded compound **8**.

The nitrobenzene **8** was reduced to aniline **9** using $Na_2S_2O_4$ in aqueous THF. An intermediate, with a structure assigned as sulfonamide **12** (Figure 1), was observed during the reaction.

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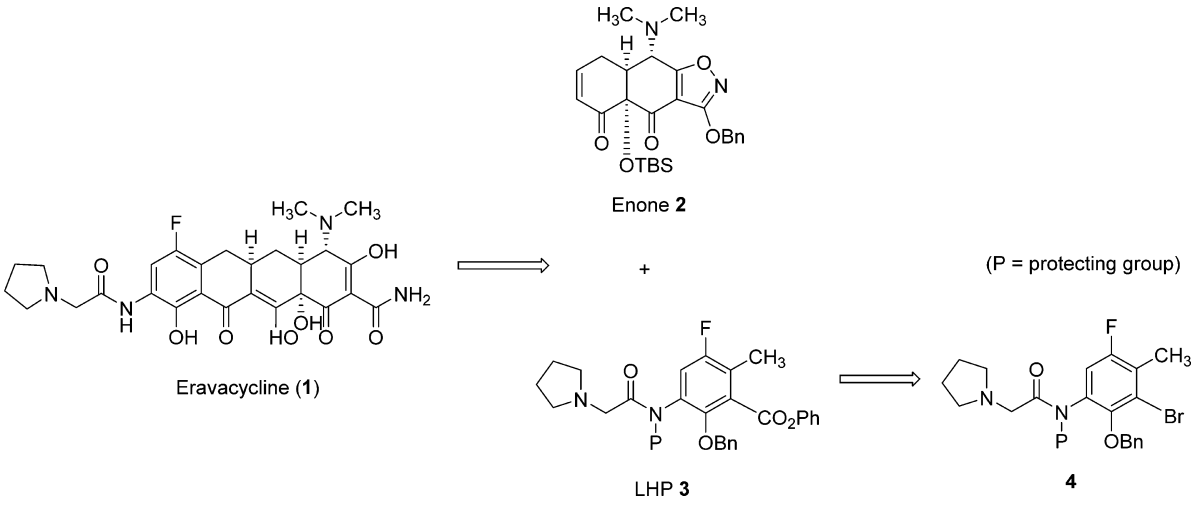
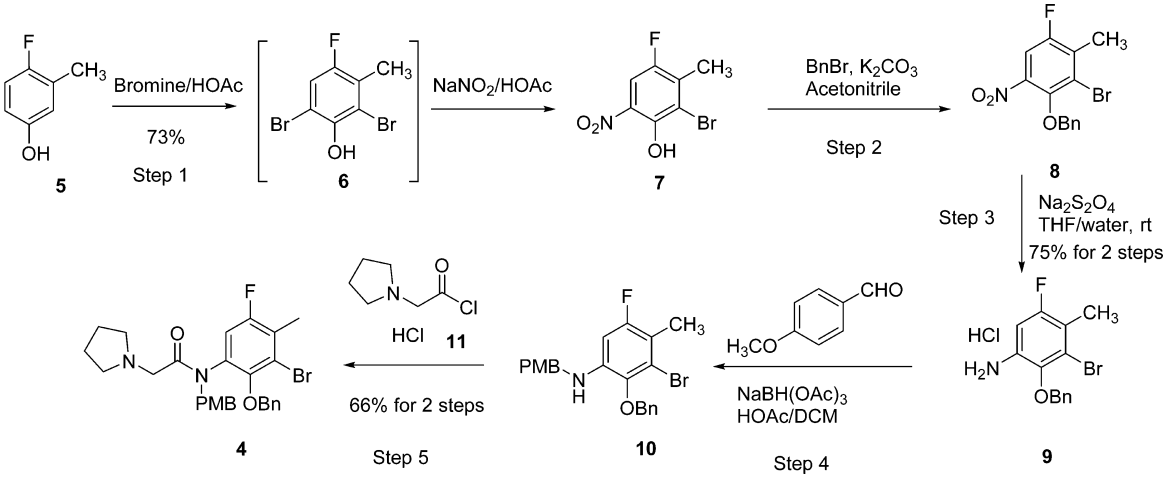
Scheme 1. Retrosynthetic Analysis of Eravacycline



Scheme 2. Synthesis of Bromide 4



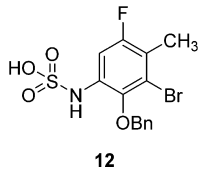
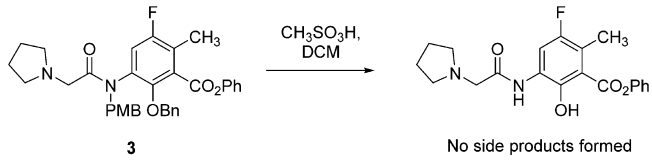


Figure 1. $Na_2S_2O_4$ reduction intermediate.

Scheme 3. Acid Removal of Protecting Groups



The intermediate **12** could be reduced to the desired aniline **9** by additional $Na_2S_2O_4$ (5 equiv total) or be hydrolyzed to **9** with hydrochloric acid. Compound **9** was purified as a hydrochloric salt by treating the solution of crude aniline **9** in TBME (10 V) with 2.5 M HCl in EtOH.

The HCl salt of compound **9** was converted to free base, and the amino group was protected with *p*-methoxybenzyl (PMB) via a reductive amination to yield **10**. PMB was selected as the *N*-protecting group as it can be more easily removed under acidic conditions than a *N*-benzyl group. Ester **3** (prepared from another route) was tested as a model compound to examine the deprotection. When treated with methanesulfonic acid (MSA) in DCM, both the *N*-PMB and *O*-benzyl groups were cleanly cleaved (Scheme 3).

The crude solution of **10** in DCM was treated with excess acyl chloride **11** (~3 equiv) at room temperature to give **4** as a hydrochloride salt, which was basified with $NaHCO_3$ and purified by flash column chromatography to afford aryl bromide **4** free base as a yellow oil.

Carboxylation To Form Fully Built LHP 3. Initially, carboxylation employing a Grignard reagent and CO_2 (Scheme 4) was studied. It required 3 equiv of *i*-PrMgCl·LiCl at 50 °C for several hours to complete the bromine−Mg exchange in THF. On the basis of an in-process analysis by LC−MS, it became clear that the acidic α proton of the amide group was removed prior to the desired bromine−magnesium exchange on the phenyl ring. After metalation was deemed complete (monitored by HPLC), CO_2 gas was bubbled into the reaction mixture. As expected, carboxylation was observed to occur at both positions but initially predominated at the side chain.[7] Once the bis-carboxylation was complete, the reaction was quenched with aqueous HCl, and then a selective decarboxylation could be achieved by heating the mixture at 45−50 °C for 2 h. After the

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Scheme 4. Carboxylation and Ester Formation

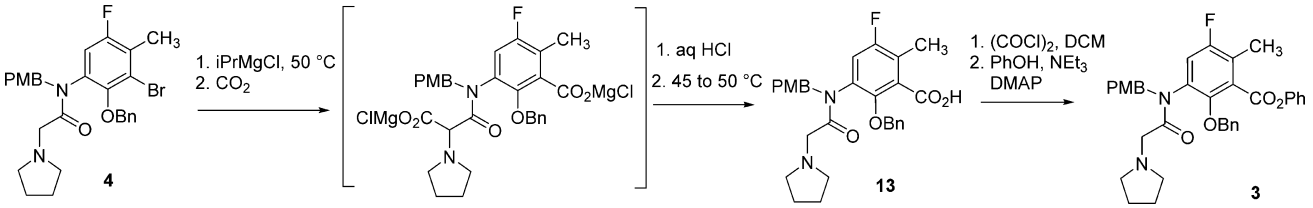
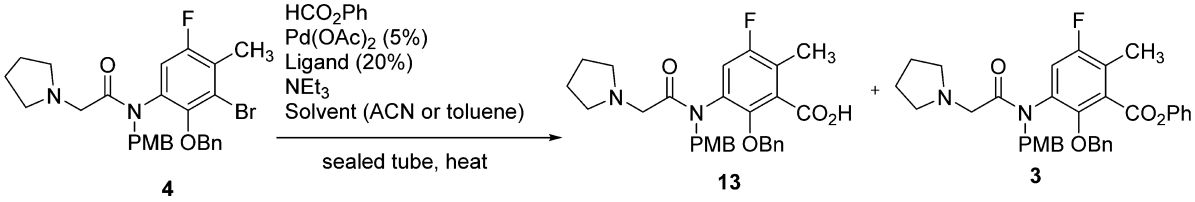


Table 1. Phenyl Carboxylation Conditions



			product ratio by HPLC area %		
entry	ligand	conditions[a]	4	13	3
1	P(*t*-Bu)$_3$·HBF$_4$	ACN, 80 °C, 24 h	29	5	51
2	Xantphos	ACN, 80 °C, 24 h	76	ND	2
3	P(*t*-Bu)$_3$·HBF$_4$	DMF, 85 °C, 24 h	88		
4	P(*t*-Bu)$_3$·HBF$_4$	MePh, 100 °C, 16 h	3	7	79
5	P(*t*-Bu)$_3$·HBF$_4$	ACN, 85 °C, 56 h	4	ND	90
6	P(*t*-Bu)$_3$·HBF$_4$	ACN, 90 °C, 45 h	6	2	86

[a]Entries 1−4 used 2 equiv of phenyl formate and 2 equiv of triethylamine; entry 5 used 3 equiv of phenyl formate and 3 equiv of triethylamine; entry 6 used 2 equiv of phenyl formate, 1 equiv of PhOH, and 3 equiv of triethylamine.

aqueous phase was adjusted to pH 6−7, product **13** precipitated out as a white solid with 98% HPLC purity. Disappointingly, the yield was only ~20%, even though the HPLC analysis of the in-process sample showed acceptable conversion, possibly due to some decomposition during the harsh condition of metalation and the ensuing reactions. Carboxylation was also attempted using PhLi and *n*-BuLi as bases. Although the metalation proceeded faster with lithium bases, the reaction gave even less of the desired carboxylation product.

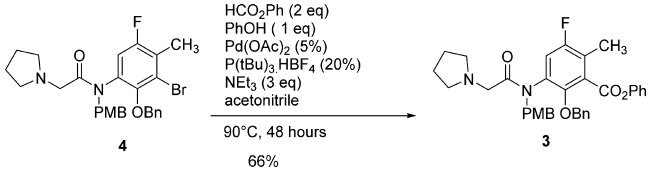
The acid **13** was treated with oxalyl chloride in DCM and the resulting acyl chloride reacted with PhOH in the presence of TEA/DMAP to afford phenyl ester **3**.

Overall, the originally proposed carboxylation/ester formation route provided access to LHP **3** but suffered from a complicated process, tedious operation, and low yield. The bis-carboxylation pathway makes the hot version of this chemistry even less attractive as it would need a large excess of ^{14}C carbon dioxide to drive the reaction to completion.

We thus turned to palladium-mediated catalytic carboxylation using phenyl formate as an alternative, which if successful, would provide the phenyl ester **3** in one step.[8] We were pleased to see that this methodology worked with this sterically hindered substrate **4** in our initial attempt. As shown in Table 1, with Pd(OAc)$_2$/P(*t*-Bu)$_3$·HBF$_4$ as catalyst, the reaction of **4** with 2 equiv of both phenyl formate and triethylamine at 80 °C in acetonitrile for 24 h provided over 50% conversion and a relatively clean reaction profile (entry 1). Using xantphos as ligand (entry 2) or DMF as solvent (entry 3) yielded only trace amounts of product. Reaction in toluene at 100 °C proceeded faster and gave a result comparable to that of the reaction in acetonitrile (entry 4). The major byproduct of this reaction is carboxylic acid **13**, which typically presents at >5%. Two approaches proved effective to minimize **13**: (a) when the amount of phenyl formate was increased from 2 to 3 equiv and with longer reaction time, no **13** was detected (entry 5); (b) addition of 1 equiv of phenol and 1 extra equiv of triethylamine to facilitate the ester formation led to a drop in the level of free acid **13** to 2% (entry 6).

Finally, the conditions used for entry 6 were adapted to prepare phenyl ester **3** employing 2 equiv of phenyl formate, 1 equiv of PhOH, and 3 equiv of triethylamine. After being stirred at 90 °C in a sealed tube for 48 h, the reaction afforded desired phenyl ester **3** in 66% isolated yield (Scheme 5).

Scheme 5. One-Pot Phenyl Carboxylation



Sampling during reaction is not recommended because CO can escape, and consequently, the reaction will stall. The cleavage of phenyl formate to phenol and CO took place in the first several hours as evidenced by HPLC analysis where no phenyl formate was detected in the in-process check (IPC) at 16 h. To resume a stalled reaction, extra phenyl formate and NEt$_3$ should be added after each sampling.

Overall, the Pd-catalyzed phenyl carboxylation reaction of **4** using phenyl formate afforded the phenyl ester **3** in a single step with satisfactory yield. It should be adaptable for the synthesis of ^{14}C-labeled LHP **3** given that ^{14}C phenyl formate is commercially available or readily prepared from ^{14}C formic acid and phenol.

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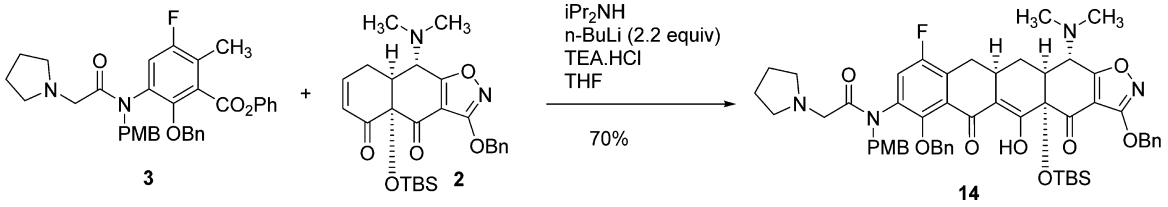
Scheme 6. Michael−Dieckmann Reaction



Scheme 7. Initial Two-Step Deprotection

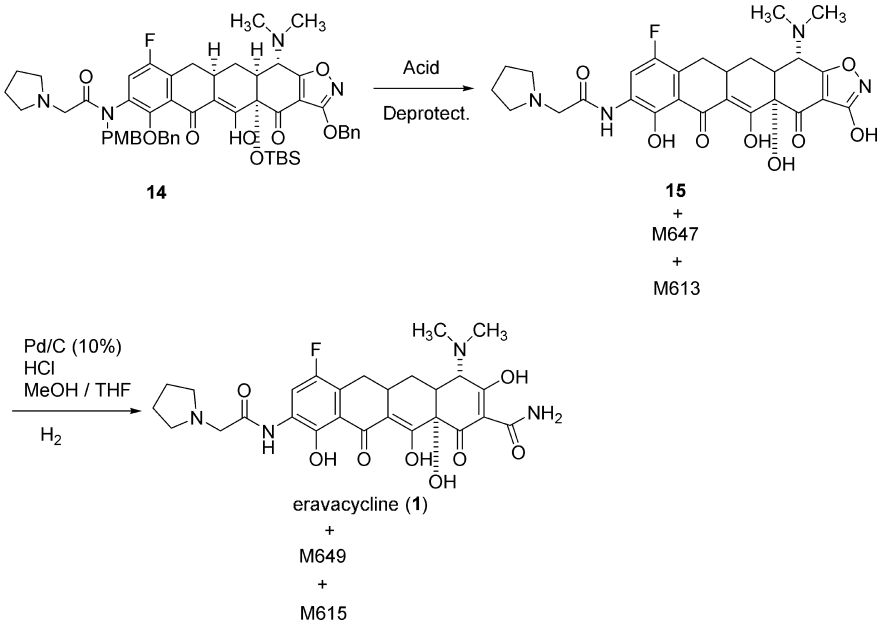


Table 2. Deprotection of PMB-Protected M−D Product under Various Conditions

			product ratio by HPLC area %		
entry	conditions	comment	**15**	M647	M613
1	MSA (10%, 15 equiv), DCM	three major products	40	11	10
2	MSA (neat, 33 equiv)	three major products	52	18	9
3	CF_3SO_3H (10 equiv), TFA/DCM	fast (2 h)	66	3	8
4	CF_3SO_3H (7 equiv), DCM	fast (2 h)	64	8	8
5	CF_3SO_3H (3 equiv), DCM	no reaction			
6	*p*-TsOH, THF/H_2O	only TBS removed			
7	CAN, THF/H_2O	fast, very messy			

Michael−Dieckmann Reaction and Global Deprotection. The Michael−Dieckmann reaction (Scheme 6) of LHP **3** and enone **2** was used to construct the core structure of eravacycline. Again due to the presence of the acidic proton α to the amide, bis-metalation consumes 2 equiv of LDA. Following our manufacturing process,[3] inverse addition of the resulting anion to the THF solution of enone **2** reproducibly afforded ~60% yield on a 2−6 g scale. While inverse addition was reliable for larger scale reactions, it was not ideal for the small-scale operation expected for ^{14}C installation. Direct addition of enone **2** to the anion solution at −78 °C produced inconsistent results due to the instability of enone and/or the Michael adduct. Adding enone to the anion solution at −100 °C proved to be more reliable and convenient on a small scale, affording 70−80% yield as determined by HPLC assay. The crude product **14** is typically obtained in ~80% HPLC purity with excess LHP **3** as the major impurity. It can be directly used in the next step without further purification since the final eravacycline will be purified by preparative HPLC.

After the Michael−Dieckmann reaction, the initial strategy was to use acid-mediated deprotection to remove the TBS, both benzyl and the PMB protecting groups, followed by catalytic hydrogenation to open the isoxazole ring (Scheme 7). A series of deprotection experiments were conducted using crude (~80% pure) Michael−Dieckmann product **14** to assess acid sources and conditions (Table 2). Unfortunately, reaction conditions that produced a significant amount of the desired **15** also gave rise to two significant side products: one with a molecular weight of 647 (product **15** + benzyl), referred to as M647, and the other with a molecular weight of 613 (product **15** + *tert*-butyl), referred to as M613.[9]

Hydrogenation of the crude product obtained from acid deprotection did not convert either side product M647 or M613 to eravacycline but instead to reduced side products with a

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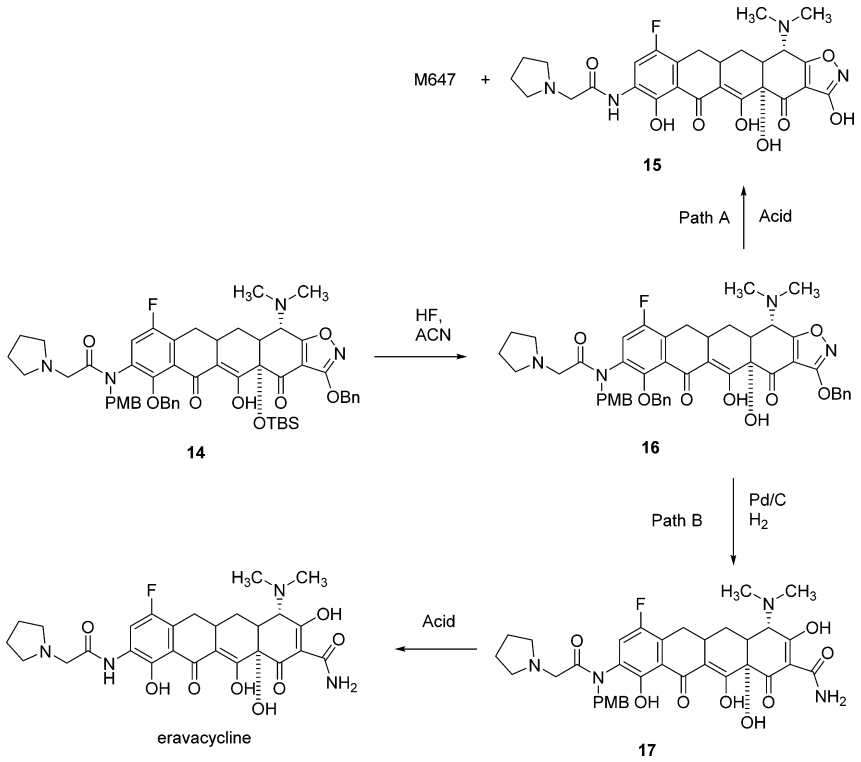
Scheme 8. Different Deprotection Pathways



molecular weight of 649 and 615, respectively (Scheme 7), referred to as M649 and M615. Minimizing side-product formation will thus be important for the subsequent chemistry.

To eliminate the *tert*-butyl source, the TBS group was removed using HF in ACN prior to strong acid treatment (Scheme 8, pathway A). As expected, the *tert*-butyl adduct M613 was not observed after acid treatment; however, the benzyl adduct M647 was still present in a significant amount (Table 3).

Table 3. Deprotection of 16 with Acid

entry	conditions	product **15** (%)	M647 (%)	M613 (%)
1	CH_3SO_3H, DCM	62	23	NA
2	CF_3SO_3H, TFA, DCM	72	6	NA

When the benzyl groups were also removed by hydrogenation prior to acid treatment (pathway B), neither impurity was observed. While this three-step deprotection sequence produced material of improved purity, it lacked efficiency and would not be ideal for C14 labeling.

Strongly acidic conditions are necessary for removal of the PMB group, but under such conditions *tert*-butyl or benzyl groups may form stabilized cations and be blamed for side-product formation. Oxidative removal of the PMB group would provide an alternative pathway for deprotection under less acidic conditions; however, the molecule was not stable to typical oxidation conditions such as ceric ammonium nitrate (Table 2, entry 7).

Precedent for such byproduct formation under acidic deprotection conditions exists in the solid-phase peptide synthesis literature.[10] Similar benzyl and *tert*-butyl migration reactions during deprotection have been noted, and in some cases, these side reactions can be prevented by addition of cation-trapping reagents such as sulfides, thiols, or silanes. A series of test reactions were run to determine the effectiveness of using dimethyl sulfide (DMS) as a cation trapping reagent for the deprotection of **14** (Table 4).

To our delight, DMS did prevent the formation of cation-derived side products. With 2.3 equiv of DMS, the side products were almost completely inhibited (entry 3), though at the expense of longer reaction time. Furthermore, it was noted that the isoxazole ring was opened as well in the presence of excess DMS. Use of 5.6 equiv of DMS can convert the product entirely to the final eravacycline (entry 4). This was not previously observed with acid-only deprotections but not surprising as DMS has been shown to act as a reducing agent under certain conditions.[11] This offers the interesting possibility of a one-pot global deprotection and reductive isoxazole opening after M−D reaction.

The drawback of using a larger excess of DMS (5.6 equiv) is that the benzyl and PMB removal are significantly slower. This can be remedied by adding DMS in two portions; the first portion (~2 equiv) is added at the beginning of the reaction as a cation trap, and the second portion added after the benzyl and PMB removal is complete (18−24 h) to accomplish reductive isoxazole ring opening. Alternatively, the reaction can be facilitated at elevated temperature. In fact, the debenzylation and isoxazole ring opening can be realized with trifluoracetic acid (TFA) and DMS. Treatment of **14** with TFA/DMS at 40 °C for 3 h removed the two benzyl groups and opened the isoxazole ring to afford an intermediate still possessing PMB and TBS (M + H = 793, monitored by LC/MS). Subsequent addition of MSA to the same pot of the reaction to remove the TBS and the PMB groups provided the desired eravacycline. When the reaction was complete, the mixture could be directly loaded on preparative HPLC after removal of volatile TFA and DMS and then eluted with acetonitrile and 0.05 N HCl to give the final product as a di-HCl salt with >96% purity (Scheme 9).

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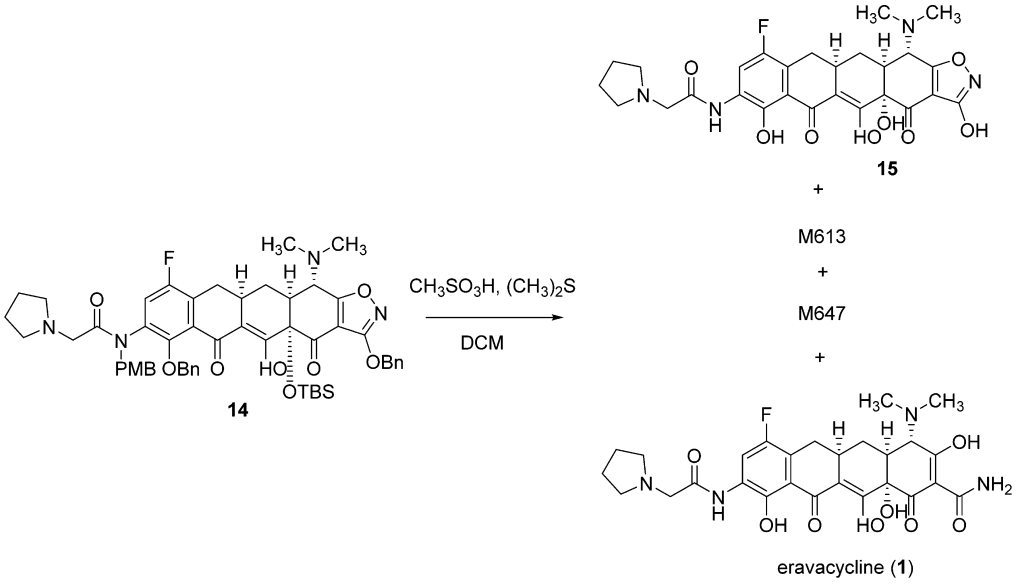
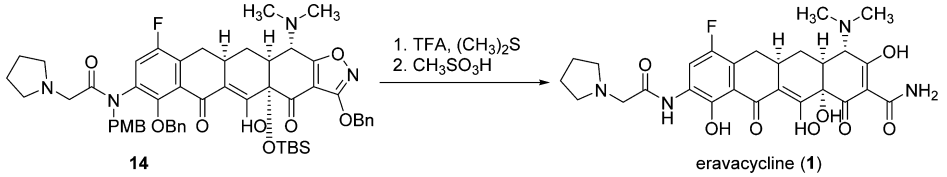
Table 4. Effect of Dimethyl Sulfide on Deprotection



entry	MSA (equiv)	DMS (equiv)	time (h)	conv (%)	(15 + 1)/(M647 + M613)	1:15
1	15	0	24	>99	3:1	1:14
2	15	0.5	24	99	87:1	1:16
3	30	2.3	24	85	>99:1	1:8
4	15	5.6	72	88	>99:1	99:1

Scheme 9. Final One-Pot Process for Global Deprotection and Isoxazole Ring Opening



It is worth noting that DMS is not as effective for cation trapping when triflic acid is used in place of MSA and completely ineffective at isoxazole ring opening in this medium. It is hypothesized that DMS is at least partially protonated by the stronger triflic acid ($pK_a \sim -14$) and not available as a nucleophile in the reaction medium.

CONCLUSION

We have developed an expedient route where eravacycline can be synthesized in eight steps from commercially available 4-fluoro-3-methylphenol and readily available enone **2**. Using this route, the ^{14}C atom can be installed in the last step of LHP **3** synthesis, and subsequently, only two chemical steps are needed to furnish the eravacycline synthesis. A high-yielding palladium-catalyzed phenyl carboxylation process was developed to potentially introduce the ^{14}C atom into the core ring system of the molecule, and a TFA/MSA/DMS system was developed to allow both the global deprotection (two benzyl groups, one PMB group, one TBS group) and reductive isoxazole ring opening to be accomplished in one pot.

EXPERIMENTAL SECTION

General Information. Solvents and reagents were obtained from commercial vendors and were used without further purification. ^1H NMR data were obtained on a 400 MHz spectrometer, while ^{13}C NMR spectra were recorded on a 100 MHz instrument. Chemical shifts are reported in δ (ppm) using residual nondeuterated solvent as an internal standard. High-resolution mass (HRMS) spectra were obtained using ESI (Q-TOF analyzer). Column chromatographic purifications were performed using prepacked silica gel cartridges.

2-Bromo-4-fluoro-3-methyl-6-nitrophenol (7). A solution of bromine (54.2 g, 339 mmol, 2.1 equiv) in acetic acid (60 mL) was added dropwise to a solution of 4-fluoro-3-methylphenol (20 g, 159 mmol, 1 equiv) in acetic acid (200 mL) over 2 h at 20−30 °C. After addition, the mixture was stirred for another 4 h, at which time the dibromination was deemed complete by HPLC. After the mixture was cooled to 0−10 °C, a solution of sodium nitrite (31.7 g, 459 mmol, 2.9 equiv) in water (90 mL) was added dropwise over 1 h at 0−10 °C. After addition, the mixture was stirred for 4 h at 20−30 °C. The mixture was then added to 320 g of crushed ice followed by a solution of sodium bisulfite (4 g, 38 mmol, 0.24 equiv) in water (160 mL), maintaining temperature at 0−10 °C. The mixture was stirred at 0−10 °C for 2−3 h for crystallization and then filtered. The filter cake was rinsed with precooled water (60 mL × 3). The filter cake was suction dried under nitrogen. The resulting crude product was dissolved in acetonitrile (55 mL). To the solution was added water (217 mL) dropwise over a period of 2 h at 20−30 °C. The mixture was cooled to 0−10 °C and stirred at this temperature for 3 h before filtration. The filter cake was rinsed with precooled water (40 mL × 3). The product was suction dried under nitrogen to give 30.5 g (73% yield) of 2-bromo-4-fluoro-3-methyl-6-nitrophenol (7) as a yellow solid with 97.2% HPLC purity: mp 70−71 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 11.06 (1H, s), 7.80 (1H, d, J = 9.2 Hz), 2.44 (3H, d, J = 2.5 Hz); ^{13}C NMR (100 MHz, CDCl3) δ 153.1 (d, J_{C-F} = 223 Hz), 149.1 (d, J_{C-F} = 1.9 Hz), 138.2 (d, J_{C-F} = 20 Hz), 131.1 (d, J_{C-F} = 10 Hz), 116.5 (d, J_{C-F} = 4.7 Hz), 109.1 (d, J_{C-F} =

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30 Hz), 16.4 (d, J_{C-F} = 2.9 Hz); HRMS *m*/*z* calcd for $C_7H_5BrFNO_3$ 248.9437; found 248.9444.

Data for the homodimer, purified as a white solid by flash chromatography on silica gel. mp: 125−127 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (1H, d, *J* = 9.2 Hz), 7.54 (1H, d, *J* = 6.0 Hz), 2.37 (3H, s), 2.26 (3H, d, *J* = 2.4 Hz); ^{13}C NMR δ; 170.7 (d,, *J* = 3.9 Hz), 157.5 (d, *J* = 248 Hz), 150.4 (d, *J* = 30 Hz), 143.9 (d, *J* = 2.8 Hz), 138.2 (d, *J* = 32 Hz), 127.1 (d, *J* = 19 Hz), 125.6 (d, *J* = 12 Hz), 123.4 (d, *J* = 7.7 Hz), 122.6 (d, *J* = 6.7 Hz), 119.5 (d, *J* = 27.7 Hz), 115.7 (d, *J* = 10.5 Hz), 107.4 (d, *J* = 220.8 Hz), 18.0, 15.6 (d, *J* = 2.9 Hz), HRMS *m*/*z* calcd for $C_{14}H_9Br_3F_2O_2$ 483.8121; found 483.8128

2-(benzyloxy)-3-bromo-5-fluoro-4-methyl-1-nitrobenzene (8). To a 100 mL flask was charged 2-bromo-4-fluoro-3-methyl-6-nitrophenol (**7**, 5.0 g, 20 mmol, 1.0 equiv) and acetonitrile (40 mL). K_2CO_3 (4.16 g, 30 mmol, 1.5 equiv) and KI (0.33 g, 2 mmol, 0.1 equiv) were added into the solution followed by BnBr (3.45g, 20 mmol, 1.0 equiv). The mixture was heated to 60−70 °C and stirred for 5 h. After the mixture was cooled, it was filtered and the filter cake was washed with acetonitrile (20 mL × 2). The filtrate was concentrated to give 2-(benzyloxy)-3-bromo-5-fluoro-4-methyl-1-nitrobenzene (**8**) which was used in the next step directly. mp: 57−59 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (1H, d, *J* = 8.9 Hz), 7.54 (2H, m), 7.42−7.34 (3H, m), 5.12 (2H, s), 2.43 (3H, d, *J* = 2.5 Hz); ^{13}C NMR δ 155.8 (d, J_{C-F} = 248 Hz), 145.9 (d, J_{C-F} = 3.4 Hz), 142.5 (d, J_{C-F} = 10.5 Hz), 135.4, 133.8 (d, J_{C-F} = 19.1 Hz), 128.8 (2 C), 128.7, 128.5 (2 C), 124.3 (d, J_{C-F} = 5.3 Hz), 111.0 (d, J_{C-F} = 29.5 Hz), 76.4, 16.0; HRMS *m*/*z* calcd for $C_{14}H_{11}BrFNO_3$ 338.9906, found 338.9923

2-(Benzyloxy)-3-bromo-5-fluoro-4-methyl-1-aminobenzene (9). To a solution of 2-(benzyloxy)-3-bromo-5-fluoro-4-methyl-1-nitrobenzene (34.0 g, 100 mmol, 1.0 equiv) in 340 mL of THF was added $Na_2S_2O_4$ (85% pure, 102.4 g, 500 mmol, 5.0 equiv) in 340 mL of water via an addition funnel. The temperature was maintained below 20 °C. The mixture was stirred with a mechanical stirrer at room temperature for 5 h, at which time HPLC showed that all nitrobenzene **8** and the intermediate **12** were consumed. To the reaction was added 340 mL of MTBE, and then the mixture was stirred for 1 h. The organic phase was separated and concentrated. The residue was dissolved in 340 mL of MTBE and then was washed with aqueous $NaHCO_3$ and brine. After layer separation, to the MTBE solution was added 2.5 M HCl in EtOH (65 mL). The resulting pale yellow slurry was filtered. The filter cake was washed with TBME (25 mL × 2) and suction dried on the funnel for 2 h and then in a high vacuum oven at 50 °C overnight to give 25.8 g (75% yield) of 2-(benzyloxy)-3-bromo-5-fluoro-4-methyl-1-aminobenzene hydrochloride (**9·HCl**) as an off-white solid: mp 203−207 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (1H, br s), 7.62 (2H, d, *J* = 7.2 Hz), 7.42−7.33 (3H, m), 7.07 (1H, d, *J* = 10.4 Hz), 4.95 (2H, s), 2.21 (3H, d, *J* = 1.8 Hz); ^{13}C NMR δ 156.5 (d, J_{C-F} = 240 Hz), 142.4, 136.6, 133.3 (br), 128.7 (2 C), 128.4 (2 C), 128.3, 121.0 (d, J_{C-F} = 7.6 Hz), 118.9 (br), 105.9 (d, J_{C-F} = 28.6 Hz), 73.9, 14.6 (d, J_{C-F} = 2.9 Hz); HRMS *m*/*z* calcd for $C_{14}H_{13}BrFNO$ 309.0165, found 309.0176

Intermediate 12: mp 182−184 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56−7.52 (2H, m), 7.44−7.34 (3H, m), 7.32 (1H, d, *J* = 12.0 Hz), 6.58 (1H, s), 4.82 (2H, s), 2.19 (3H, d, *J* = 1.8 Hz); ^{13}C NMR δ 156.8 (d, J_{C-F} = 238 Hz), 139.9 (d, J_{C-F} = 2.9 Hz), 136.6, 136.3 (d, J_{C-F} = 13.3 Hz), 128.5 (2 C), 128.3, 128.20 (2 C), 119.2 (d, J_{C-F} = 8.6 Hz), 114.9 (d, J_{C-F} = 21 Hz), 103.9 (d, J_{C-F} = 29.6 Hz), 73.9, 14.2 (d, J_{C-F} = 2.8 Hz); HRMS *m*/*z* calcd for $C_{14}H_{13}BrFNO_4S$ 388.9733, found 388.9733.

Reductive Amination. A 500 mL RBF was charged with 17.3 g (50 mmol, 1.0 equiv) of aniline hydrochloride salt (**9·HCl**), 100 mL of water, 50 mL of saturated $NaHCO_3$, and 200 mL EtOAc. The suspension was stirred at room temperature for 2 h until all of the solids had dissolved. The layers were separated, and the organic layer was washed with saturated $NaHCO_3$ (50 mL × 2) and brine (50 mL × 1) and dried over Na_2SO_4. After filtration, the filtrate was concentrated, and the residual was further dried under high vacuum for 1 h to give 15.6 g of yellow solid. The solid was dissolved in 150 mL DCM. To the solution was charged *p*-methoxybenzaldehyde (7.21g, 55 mmol, 1.1 equiv), HOAc (3.3 g, 55 mmol, 1.1 equiv), and $NaBH(OAc)_3$ (14.8 g, 70 mmol, 1.4 equiv). The resulting solution was stirred under nitrogen atmosphere at room temperature for several hours until HPLC showed the starting material was fully consumed. The reaction was quenched with 50 mL of water and diluted with 100 mL of DCM. After gas evolution ceased, the mixture was transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_3$ (50 mL × 2) and brine (150 mL) and dried over Na_2SO_4. The filtrate, a beige color solution, assuming quantitative yield from aniline HCl, was used for the next step without further purification. A fraction of the solution was purified by flash chromatography (eluted with EtOAc in hexanes 10−100%) to give the pure product (**10**) as a white solid: mp 86−87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (2H, d, *J* = 6.7 Hz), 7.43−7.34 (3 H, m), 7.25 (2H, d, *J* = 8.5 Hz), 6.87 (2H, d, *J* = 8.5 Hz), 6.42 (1H, d, *J* = 12.2 Hz), 6.09 (1H, NH, br t, *J* = 6.0 Hz), 4.86 (2H, s), 4.26 (2H, d, *J* = 6.0 Hz), 3.71 (3H, s), 2.15 (3H, d, *J* = 1.2 Hz); ^{13}C NMR δ 157.3 (d, J_{C-F} = 236 Hz), 158.1, 141.4 (d, J_{C-F} = 12.4 Hz), 138.7 (d, J_{C-F} = 2.9 Hz), 136.9, 131.2, 128.4 (2 C), 128.2 (2 C), 128.2 (2 C), 128.0, 119.8 (d, J_{C-F} = 8.6 Hz), 113.8 (2 C), 110.0 (d, J_{C-F} = 20.0 Hz), 97.7 (d, J_{C-F} = 28.6 Hz), 73.0, 55.0, 45.3, 13.9 (d, J_{C-F} = 2.9 Hz); HRMS *m*/*z* calcd for $C_{22}H_{21}BrFNO_2$ 429.0740, found 429.0751.

Acylation. The solution obtained from previous step (assuming 50 mmol, 21.5 g of **10** in 200 mL DCM, 1.0 equiv) was cooled to 10 °C with an ice−water bath. To the solution under nitrogen atmosphere was added 1-pyrrolidineacetyl chloride hydrochloride (**11**, 18.4 g, 100 mmol, 2.0 equiv) over 5 min. The suspension was stirred at 10 °C for 30 min and then was warmed to room temperature and stirred for another 2 h. A second portion of **11** (11.0 g, 59.8 mmol, 1.2 equiv) was added, and the mixture was stirred overnight at which time the reaction was deemed complete by HPLC. The reaction was quenched with water. The organic layer was washed with saturated $NaHCO_3$ (50 mL × 2) and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (eluted with EtOAc in hexanes 10−100%) to afford 17.5 g (65% yield for two steps) of product **4** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.30 (5H, m), 7.12 (2H, d, *J* = 8.5 Hz), 6.74 (2H, d, *J* = 8.5 Hz), 6.55 (1H, d, *J* = 9.5 Hz), 5.43 (1H, d, *J* = 14.7 Hz), 4.84 (1H, d, *J* = 9.8 Hz), 4.56 (1H, d, J = 9.8 Hz), 4.16 (1H, d, d, *J* = 14.7 Hz), 3.72 (s, 3H), 3.17 (1H, d, *J* = 15.3 Hz), 3.11 (1H, d, *J* = 15.3 Hz), 2.55−2.40 (4H, m), 2.33 (3H, d, *J* = 2.4 Hz), 1.80−1.64 (4H, m); ^{13}C NMR δ 169.8, 159.0, 156.4 (d, J_{C-F} = 245 Hz), 148.4 (d, J_{C-F} = 3.8 Hz), 135.9, 134.0 (d, J_{C-F} = 11.4 Hz), 130.3 (2 C), 129.4, 128.5 (2 C), 128.4, 128.3 (2 C), 127.3 (d, J_{C-F} = 19.1 Hz), 122.3 (d, J_{C-F} = 6.7 Hz), 115.3 (d, J_{C-F} = 24.8 Hz), 113.7 (2 C), 75.08, 57.3, 55.1, 54.0 (2 C), 51.4, 23.6 (2 C), 15.3 (d, J_{C-F} = 2.9 Hz); HRMS *m*/*z* calcd for $C_{28}H_{30}BrFN_2O_3$ 540.1424, found 540.1423.

Phenyl Carboxylation To Prepare 3. To a 10 mL reaction tube with stirring bar were added bromide **3** (270 mg, 0.5 mmol, 1.0 equiv), $Pd(OAc)_2$ (5.6 mg, 0.025 mmol, 0.05 equiv), P(*t*-Bu)$_3$·HBF$_4$ (29.1 mg, 0.1 mmol, 0.20 equiv), phenol (47 mg, 0.5 mmol, 1.0 equiv), acetonitrile (1.0 mL), phenyl formate (109 μL, 1 mmol, 2.0 equiv), and triethylamine (208 μL, 1.5 mmol, 3.0 equiv). The resulting suspension was sealed, protected with nitrogen (vacuum and nitrogen, three cycles), and stirred in a 90−95 °C oil bath for 48 h. The reaction mixture was cooled to room temperature and concentrated. The residue was purified by flash chromatography (eluted with EtOAc in hexane 10−100%) to afford 192 mg (66% yield) of **3** as a thick oil, which solidified during storage: mp 82−84 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.33 (7H, m), 7.25 (1H, dd, *J* = 7.2, 7.2 Hz), 7.16 (2H, d, *J* = 8.5 Hz), 7.00 (2H, d, *J* = 8.0 Hz), 6.77 (2H, d, *J* = 9.2 Hz), 6.65 (1H, d, *J* = 9.5 Hz), 5.59 (1H, d, *J* = 14.0 Hz), 4.92 (1H, d, *J* = 9.8 Hz), 4.78 (1H, d, J = 9.8 Hz), 4.17 (1H, d, d, *J* = 14.0 Hz), 3.75 (s, 3H), 3.30 (1H, d, *J* = 15.9 Hz), 3.16 (1H, d, *J* = 15.9 Hz), 2.65−2.45 (4H, m), 2.35 (3H, d, *J* = 2.4 Hz), 1.79−1.69 (4H, m); ^{13}C NMR δ 169.9, 165.0 (d, J_{C-F} = 2.8 Hz), 159.0, 156.4 (d, J_{C-F} = 244 Hz), 150.2, 147.8 (d, J_{C-F} = 2.9 Hz), 135.9, 133.5 (d, J_{C-F} = 10.5 Hz), 131.1 (d, J_{C-F} = 5.7 Hz), 130.4 (2 C), 129.5 (2 C), 129.2, 128.6 (2 C), 128.49, 128.46 (2 C), 126.4, 123.9 (d, J_{C-F} = 20.0 Hz), 121.4 (2 C), 118.1 (d, J_{C-F} = 24.8 Hz), 113.8 (2 C), 76.8, 57.6, 55.2, 54.1 (2 C), 51.1, 23.6 (2 C), 11.8 (d, J_{C-F} = 2.9 Hz); HRMS *m*/*z* calcd for $C_{35}H_{35}FN_2O_5$ 582.2530, found 582.2529

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Carboxylic acid (**13**) from Grignard reaction: mp 161−163 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.36−7.25 (5H, m), 7.18 (2H, d, J = 8.5 Hz), 6.84 (2H, d, J = 8.5 Hz), 6.78 (1H, d, J = 9.8 Hz), 5.01 (1H, d, J = 9.8 Hz), 4.87 (1H, d, J = 14.0 Hz), 4.68 (1H, d, J = 14.0 Hz), 4.39 (1H, d, J = 16.5 Hz), 3.93 (1H, d, J = 10.4 Hz), 3.82 (1H, d, J = 16.5 Hz), 3.73 (3H, s), 3.10 (4H, m), 2.27 (3H, d, J = 2.5 Hz), 1.85 (4H, m); ^{13}C NMR δ173.4 (d, J_{C-F} = 2.9 Hz), 167.1, 161.1, 158.4 (d, J_{C-F} = 242 Hz), 146.1 (d, J_{C-F} = 2.9 Hz), 141.3 (d, J_{C-F} = 3.8 Hz), 138.3, 132.2 (d, J_{C-F} = 10.5 Hz), 131.9 (2 C), 130.0, 129.9 (2 C), 129.4 (2 C), 129.3, 123.9 (d, J_{C-F} = 19.1 Hz), 115.2 (2 C), 114.1 (d, J_{C-F} = 25.7 Hz), 77.2, 57.3, 55.7, 55.5 (2 C), 53.5, 24.0 (2 C), 12.0 (d, J = 3.9 Hz); HRMS m/z calcd for $C_{29}H_{31}FN_2O_5$ 506.2217, found 506.2219.

Michael−Dieckmann Reaction To Form 14. To a 25 mL flame-dried round-bottom flask under nitrogen was charged triethylamine hydrochloride (6 mg, 0.044 mmol, 0.005 equiv) and 3 mL of anhydrous THF followed by diisopropylamine (350 μL, 2.5 mmol, 2.6 equiv). The reaction mixture was cooled to −78 °C and a 2.5 M solution of n-BuLi in THF (880 μL, 2.2 mmol, 2.3 equiv) was added over 10 min. After addition, the reaction mixture was warmed to −15 °C, stirred for 30 min, then recooled to −75 °C. A solution of LHP **3** (582 mg, 1 mmol, 1.05 equiv) in 2 mL of THF was added via a syringe over 5 min into the reaction mixture while the temperature was maintained between −73 and −75 °C. After addition, the reaction was stirred at −75 °C for an additional 15 min. The bath was switched to liquid nitrogen/EtOH, and the reaction mixture was cooled to −100 °C. A solution of enone **2** (458 mg, 0.95 mmol, 1.0 equiv) in 2 mL of THF was added via a syringe over 3 min while the temperature was maintained at −100 to −95 °C. After addition, the reaction was stirred for an additional 15 min, and then the bath was removed. The reaction was warmed to −20 °C and maintained at −20 to −15 °C for 15 min (HPLC monitoring confirmed that all uncyclized species were consumed). The reaction was quenched with 3 mL of saturated NH_4Cl. The mixture was diluted with 10 mL of water and extracted with EtOAc (10 mL × 2). The combined organics were washed with 1 N NaOH (10 mL × 2, to remove PhOH) and 10 mL of saturated NH_4Cl, dried over Na_2SO_4, and filtered, and the filtrate was concentrated. The crude product was subjected to flash column (eluted with DCM/MeOH) to give 678 mg (yield 70%) of compound **14** as a pale yellow solid. Purity: 90% (containing ∼9% of residual **3**).

For characterization, the free base of **14** was further purified by chromatography (eluted with EtOAc containing 0.2% of NEt_3 and hexane, 50 to 100%). Because of the large protecting groups (i.e., one TBS, two benzyl, and one PMB groups) in the molecule, two rotatomers were observed by ^{1}H NMR with a ratio of approximately 0.4−0.6 based on the ^{1}H NMR integration: mp 65−67 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 15.95 (1H, s), 7.49 (2H, d, J = 7.9 Hz), 7.4 to 7.3 (8H, m), 7.16 (0.8H, d, J = 8.5 Hz), 7.06 (1.2H, d, J = 8.6 Hz), 6.77 (1.2H, d, J = 8.6 Hz), 6.74 (0.6H, d, J = 8.6 Hz), 6.73 (0.8H, d, J = 8.4 Hz), 6.67 (0.4H, d, J = 8.6 Hz), 5.65 (0.4H, d, J = 14.0 Hz), 5.45 (0.6H, d, J = 14.0 Hz), 5.36 (2H, s), 4.85 (1H, m), 4.68 (1H, m), 4.12 (0.4H, d, J = 14.0), 3.94 (1H, d, J = 11.0 Hz), 3.75 (0.6H, d, one peak is buried under the methyl signals), 3.744 and 3.735 (3H, s and s), 3.30 to 2.92 (3H, m), 2.70 to 2.40 (8H, m), 2.49 (6H, s), 2.15 (1H, d, J = 14.7 Hz), 1.80 to 1.66 (4H, m), 0.84 and 0.83 (9H, s and s), 0.274 and 0.267 (3H, s and s), 0.16 and 0.15 (3H, s and s); For ^{13}C NMR, the rotatomer signals from the same carbon are listed in the brackets. ^{13}C NMR δ 186.99 (187.02), 182.8 (183.4), 182.2 (181.8), 181.7 (181.6), 169.9 (169.6), 167.5, 159.1 (158.9), 154.1 (d, J_{C-F} = 243.2 Hz) [154.0 (d, J_{C-F} = 243.2 Hz)], 152.8 (d, J_{C-F} = 2.9 Hz) [151.2 (d, J_{C-F} = 2.9 Hz)], 136.2 (d, J_{C-F} = 9.5 Hz) [135.6 (d, J_{C-F} = 10.5 Hz)], 135.9, 135.8, 134.9, 130.3 (130.2, 2C), 129.6, 129.4, 129.3, 129.0, 128.95, 128.7, 128.54, 128.48, 128.46 (peaks from 129.6 to 128.5 ppm overlap and may represent multiple carbons, 126.1 (d, J_{C-F} = 4.8 Hz) [126.0 (d, J_{C-F} = 5.7 Hz)], 120.9 (d, J_{C-F} = 21.9 Hz) [121.4 (d, J_{C-F} = 24.8 Hz)], 113.8 (d, J_{C-F} = 14.3 Hz), 108.4, 107.5, 81.65 (81.71), 77.2, 72.6, 61.2, 57.2 (57.5), 55.2, 54.1, 53.8, 51.7 (50.7), 46.4, 41.8, 30.9 (31.0), 27.9, 26.0, 23.6 (2C), 22.6, 19.1, −2.5, −3.6 (−3.7) ppm; HRMS m/z cacld for $C_{55}H_{63}FN_4O_9Si$ 970.4348, found 970.4296.

Global Deprotection and Reduction. Compound **14** (126 mg, 0.13 mmol, 1.0 equiv) containing ∼9% of LHP was dissolved in a mixture of TFA (1.6 mL) and DMS (0.32 mL) and the mixture heated for 3 h at 40 °C. The mixture was then treated with MSA (0.16 mL), and the heating was continued for 20 h at which time HPLC indicated all intermediates were converted to the desired eravacycline. The solvent was removed by concentration, and the residual was dissolved in 2 mL of 0.05 N HCl and acetonitrile (2:1) and subjected to prep HPLC purification. The desired fraction was collected, acetonitrile was removed by rotavapor, and the remaining solution was freeze-dried to give 51 mg (yield 62%) of eravacycline di-HCl salt (**1**) as a yellow solid: purity 97.9%; mp 197−199 °C dec. The spectral data matched those from original sample as reported in our previous publication.[3]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02442.

^{1}H and ^{13}C NMR spectra for all new compounds and eravacycline (PDF)

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Notes

The authors declare no competing financial interest.

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Asymchem Life Science (Tianjin) developed the process to make intermediate **9**.

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