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# Synthesis development of an aminomethylcycline antibiotic via an electronically tuned acyliminium Friedel–Crafts reaction

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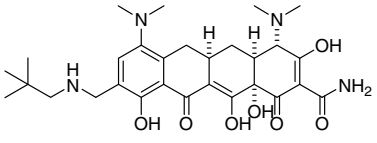
ABSTRACT

With the goal of improving the synthetic efficiency, the development of a convergent synthesis of a minocycline derivative PTK0796 via an intermolecular acyliminium Friedel–Crafts reaction (Tscherniac–Einhorn reaction) is described. The entire C9 neopentylaminomethyl side chain was installed in one step using an electronically optimized chloromethylacyliminium precursor in 83% yield. Deprotection and re-equilibration to the C4 α-epimer in the presence of $CaCl_2$ and ethanolamine or NaOH afforded the target aminomethylcycline antibiotic. The corresponding crystalline tosylate salt was found to exhibit improved solid state stability.



The tetracyclines comprise a family of antimicrobials which have been in clinical use for over 50 years and include tetracycline, doxycycline, and minocycline.[1] As a class, they have a broad spectrum of antimicrobial activity, including gram-positive, gram-negative, anaerobic, and atypical bacteria. Recently, their clinical use has declined, primarily as a result of the increased prevalence of tetracycline resistance and availability of effective alternative therapies. Due to the rising incidence of multi-drug resistant bacteria, there is a growing need for the development of new antibacterials that are effective against these organisms.[2] Despite intense effort, very few of these new antibiotics have been approved by the FDA in the past decade, and even fewer show oral activity.[3]



**PTK0796**

PTK0796, discovered by Paratek, is a novel semi-synthetic antibiotic in the tetracycline class known as the aminomethylcyclines containing a neopentylaminomethyl group attached at the C9 position.[4] PTK0796 represents a significant advance within this class of antibiotics due to its activity against tetracycline resistant organisms with either ribosomal protection and/or efflux mechanism of resistance. In vitro studies have shown it to have activity against the vast majority of gram-positive pathogens, including multi-drug resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci*.[5] It has also shown improved activity over minocycline against a wide spectrum of gram-negative and anaerobic clinical bacterial isolates. Both oral and intravenous formulations were developed for clinical testing.[6]

The current synthesis of PTK0796, depicted in Scheme 1, has been used for multi-kilogram preparations. In step 1, minocycline hydrochloride was reacted with nearly three equivalents of hydroxymethylphthalimide in triflic acid. Compound **1** possesses several reactive function groups, the C1 primary amide being more reactive than C9 or the C10 phenol. Due to this fact, the reaction afforded a mixture of bis- and tris-adducts **3** and **4**. In step 2, the phthalimides were deprotected with large excess of methylamine to afford a mixture of mono- and bis-aminomethyl **5** and **6**, along with solid waste phthalamide **7**. Compound **5** was found to be unstable and required storage at sub-ambient temperatures. In step 3, de-aminomethylation of the primary amide and a concomitant reductive amination of C9 methylamine with pivaldehyde afforded crude PTK0796. After reverse phase chromatographic purification, pH adjustment and precipitation afforded PTK0796 as an amorphous, unstable solid. The overall crude yield of PTK0796 was about 35%, and 50% was recovered after the purification.

For a long-term manufacturing route, the following issues needed to be addressed: (1) the phthalimide protection and deprotection, (2) instability of aminomethyl intermediate **5**, (3) chromatographic purification, and (4) the amorphous nature of the final

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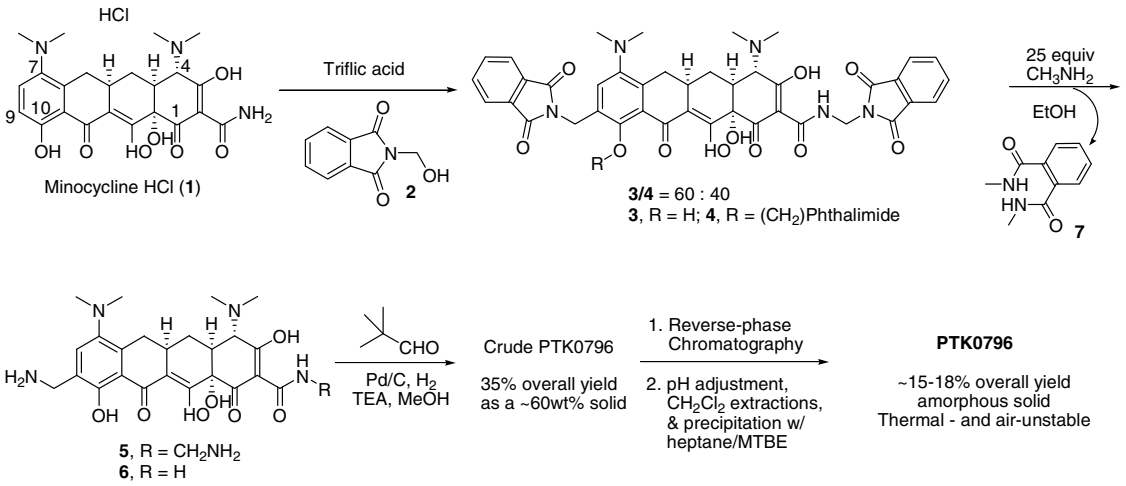


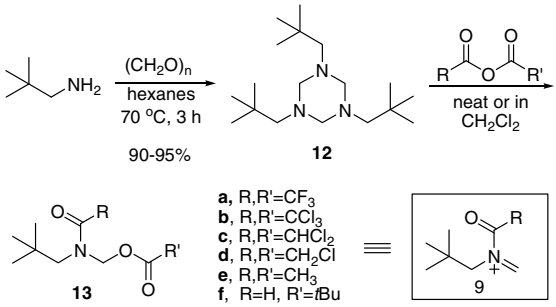

**Scheme 1.** Current synthesis of PTK0769.

product. We envisioned that a direct method of installing the entire C9 side chain that should address the first two issues. For the later two issues, we adopted a salt screening strategy, hoping to find a crystalline salt of PTK0796 that would simplify purification and improve stability.

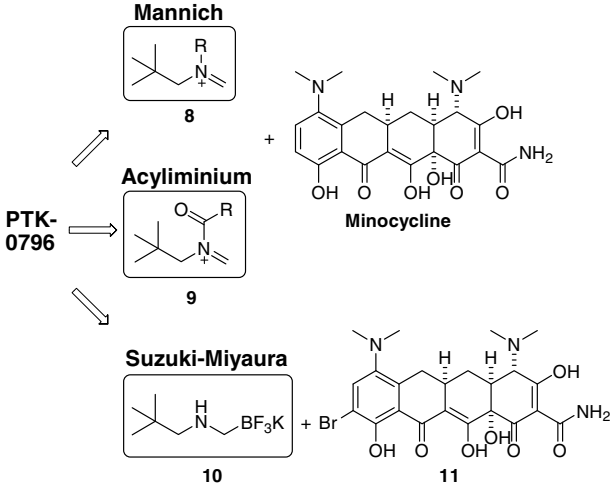
Three strategies were explored for installing the entire C9 side chain (Scheme 2): (1) Mannich reaction of minocycline with iminium ion **8**; (2) Tscherniac–Einhorn reaction of minocycline with acyliminium **9**; and (3) Suzuki–Miyaura cross-coupling of 9-bromo-minocycline (**11**) with trifluoroborate **10**.

In the Mannich route, the reactions of minocycline with several iminium ions and precursors of **8** were investigated under a variety of reaction conditions.[7] The outcomes were either no reaction, hydroxymethylation of the primary amide, C4 epimerization, or decomposition. In all cases, no or only trace desired product was detected. Similarly, attempted Suzuki cross-coupling of **10** with either 9-bromo-minocycline (**11**) or bromobenzene afforded no desired products.[8] On the other hand, the use of acyliminium ion **9** derived from neopentylamine offered promising results.

Acyliminium precursors **13** were conveniently prepared in two steps starting from neopentylamine and paraformaldehyde to afford triazane **12**,[9] followed by treatment with anhydrides (Scheme 3).[10] The reactivity of the later reaction increases with increasing electron-withdrawing ability of the anhydride R group. The identities of crude compounds **13a–f** were confirmed by NMR, and each was used directly in the next step without purification.

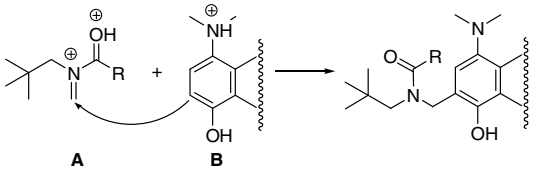



**Scheme 3.** Preparation of acyliminium precursors **13**.

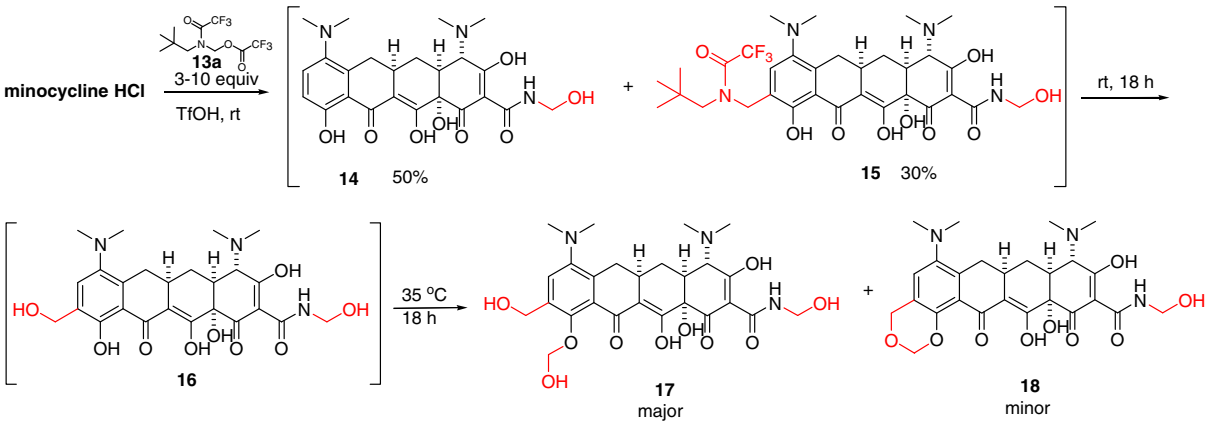



**Scheme 2.** Synthetic approaches to PTK0796.

While solvents for the Tscherniac–Einhorn reaction were not extensively screened, triflic acid proved to be a superior solvent relative to methanesulfonic acid or sulfuric acid in yield and purity. Triflic acid solutions of minocycline and PTK0796 are stable to air oxidation, C4 epimerization, and other modes of degradation. More importantly, triflic acid also protonates[11] acyliminium ions to generate a super-electrophilic species **A** that promotes efficient reaction with an electronic deficient, positively charged D-ring of minocycline (**B**).




The formation of acyliminium precursor **13a** from **12** with trifluoroacetic anhydride was complete within 10 min at 22 °C based on NMR spectroscopic analysis. It was used directly in the reaction with minocycline HCl in triflic acid at 22 °C, and quickly afforded a mixture of hydroxymethylated amide **14** and desired **15** (Scheme 4) based on LC/MS and NMR analysis. Further aging at 22 °C led to disappearance of **15** and the formation of bis-hydroxymethyl adduct **16**. Upon warming to 35 °C, **16** was transformed to tris-hydroxymethylated **17** and the cyclic ether **18**. A similar outcome

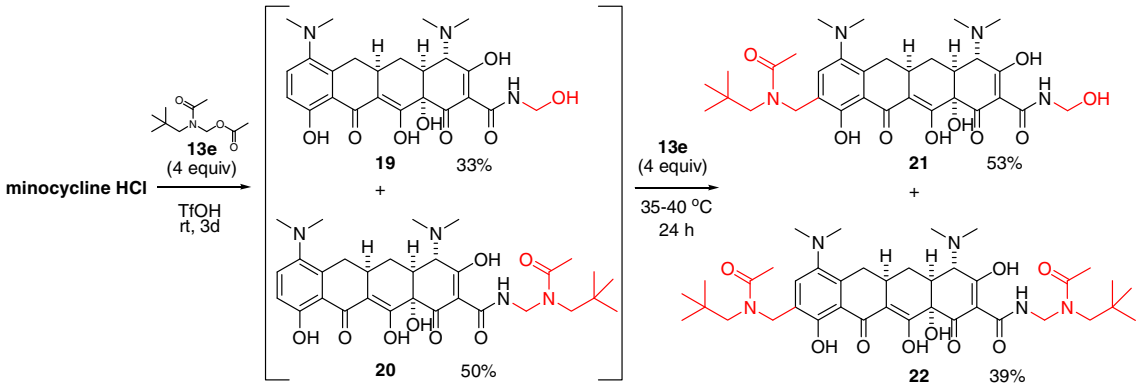



**Scheme 4.** Tscherniac–Einhorn reaction of minocycline with **13a**.

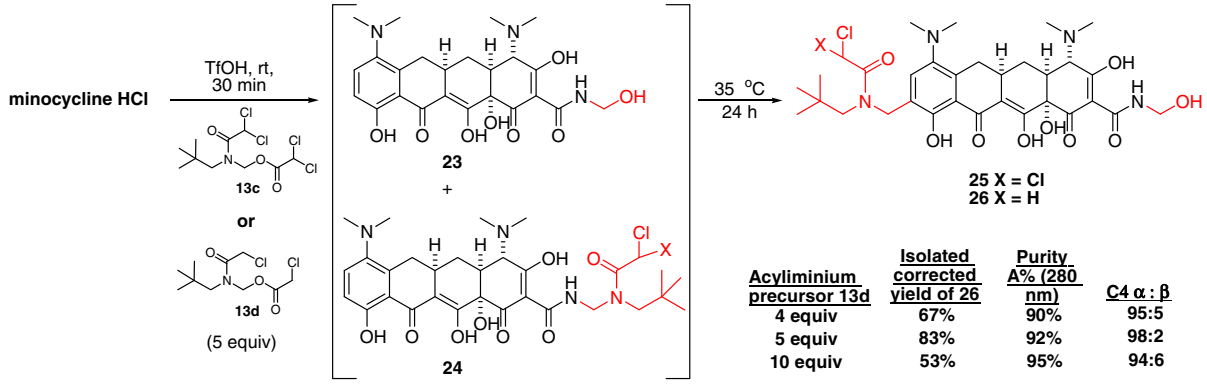
was also observed with **13b**. These results indicated that these highly electronically deficient trifluoroacetamide and trichloroacetamide groups are good leaving groups, and are readily displaced by water presumably via quinone methide intermediates.

Hoping to suppress the leaving-group problem, we then examined electronically neutral acyliminium precursor **13e** which was prepared from **12** with acetic anhydride at 100 °C for 1 h. In the subsequent reaction with minocycline HCl in triflic acid at 22 °C, the reaction slowly produced a mixture of primary amide functionalized **19** and **20** (Scheme 5). Further aging at 35–40 °C with additional **13e** afforded a mixture of desired C9 functionalized **21** and **22** in 92% combined yield. As expected with acetamide not being a good leaving group, no bis-hydroxylated **16** was detected. Thus, **13e** was significantly less reactive than **13a/b**, and the amide groups at C9 in the resulting intermediates are not displaced by water nor were hydroxymethylene byproducts at C10 —OH observed.

Since neither electron-deficient **13a/b** nor electron-neutral **13e** provided the desired reactivity profile, so screening was continued with electronically attenuated dichloro and monochloro acyliminium precursors **13c** and **13d**. These compounds were prepared from **12** and dichloroacetic anhydride or chloroacetic anhydride, respectively, at 20–35 °C for 20 min and used directly without isolation in the reaction with minocycline in triflic acid (Scheme 6). In contrast to the above studies, each of these reactions produced a single product, **25** or **26**, respectively. Optimized yield (83% assay




**Scheme 5.** Tscherniac–Einhorn reaction of minocycline with **13e**.

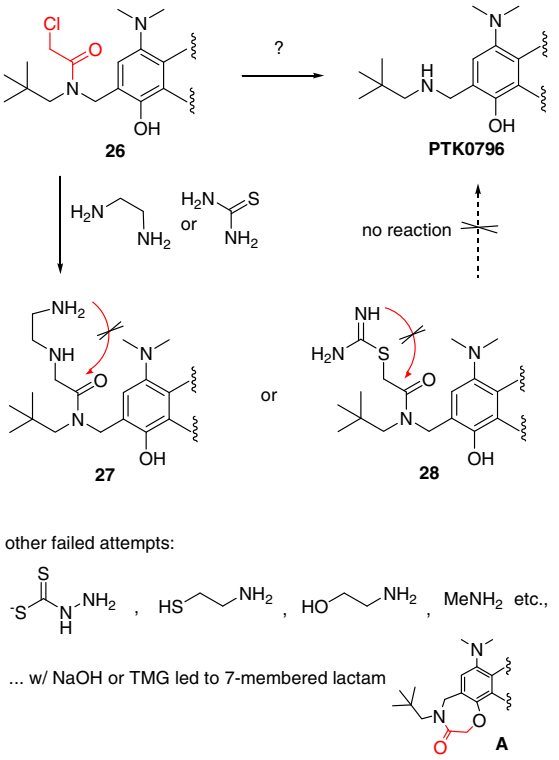



| Acyliminium precursor 13d | Isolated corrected yield of 26 | Purity A% (280 nm) | C4 α : β |
|---|---|---|---|
| 4 equiv | 67% | 90% | 95:5 |
| 5 equiv | 83% | 92% | 98:2 |
| 10 equiv | 53% | 95% | 94:6 |

**Scheme 6.** Tscherniac–Einhorn reaction of minocycline with **13c** or **13d**.

yield on the isolated crude) and purity were achieved using 5 equiv of **13c** or **13d** at 35–40 °C for 24 h. Thus, a one-step installation of the entire C9 side chain was achieved.

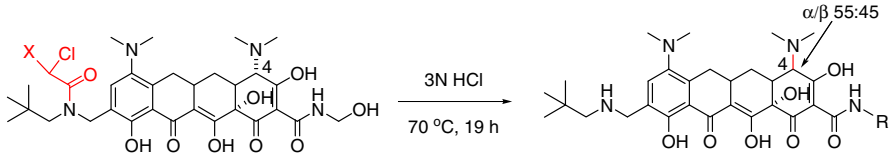
With compounds **25** and **26** in hand, the removal of the (di)chloroacetyl and hydroxymethyl groups remains to complete the synthesis. It is known that the chloroacetyl group could be cleaved by an intramolecular- assisted removal strategy, such as with thiourea[12] or hydrazinedithiocarbonate.[13] However, all attempts on **26** with these and other mono- and bi-functional nucleophiles resulted in clean displacement of the chloride (e.g., **27**, **28**), but no subsequent inter- or intramolecular cleavage of the acetyl group (Scheme 7). Examination of molecular models suggests that the congested steric nature of the region near the amide carbonyl prohibited proper alignment of the reactive centers even in the intramolecular sense. Additional attempts to cleave the dichloroacetyl group in **25** by treatment with tertiary amines bases[14] and other methods did not afford any desired product either.




**Scheme 7.** Attempted deprotection of compound **26**.

Due to problems with the assisted removal strategy deprotection, further studies focused on **26** under conventional hydrolysis conditions. Interestingly, simple heating of **26** in 3 N HCl at 70 °C afforded PTK0796, in which both the on the C2 amide hydroxymethyl on the C2 amide and the chloroacetyl groups were cleaved, as well as small amounts of hydroxymethyl **29**. However, the C4 center was epimerized to a 55:45 α/β mixture (Scheme 8). Under the same hydrolysis conditions, dichloride **25** hydrolyzed about two times slower. Two other aqueous acids, 3 N TfOH and 4.5 N $H_2SO_4$, were also investigated in the hydrolysis of **26**, and found to be inferior to 3 N HCl with respect to yield and dehydroxymethylation.

Applying Noseworthy's back-epimerization protocol for tetracycline,[15] a 55:45 PTK0796 C4 α/β mixture was back-epimerized to a 92:8 α/β mixture by heating at 105 °C in aqueous *n*-butanol in the presence of $CaCl_2$ and ethanolamine. Likewise, PTK0796 that was rapidly epimerized to a 22:78 αβ mixture by dissolution in acetic acid was re-equilibrated to a 92:8 mixture with similar efficiency (Scheme 9). Subsequently, it was found that ethanolamine could be replaced by NaOH, which allowed hydrolysis and epimerization to be carried out in a one-pot process. Normally, tetracyclines are sensitive to strong bases, but presumably, the compound is stabilized by chelation to $CaCl_2$, attenuating the destructive effect of NaOH. Thus, after the completion of the HCl hydrolysis of **26** in the presence of $CaCl_2$, the mixture was directly basified with NaOH and subjected to the back-epimerization conditions in the same pot (Scheme 10). The overall assay yield of crude PTK0796 for the two steps was 45% (67% for each step).




**26:** X = H
**25:** X = Cl

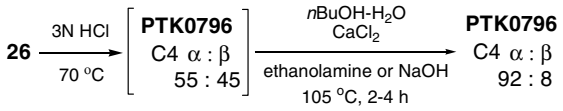
**>95% conversion**
**50% conversion**

**PTK0796:** R = H
**29:** R = $CH_2OH$

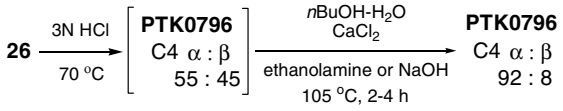
**26** —Aq. Acid, 70 °C, 24 h→ **PTK0796 (C4 α/β epimer)** + **29 (C4 α/β epimer)**

| Aqueous acid | HPLC yield of PTK0796 epimers | ratio of PTK0796 : 29 (C4 α/β epimers) |
|---|---|---|
| 3 N TfOH | 47% | 50 (55:45) : 50 (55:45) |
| 4.5 N $H_2SO_4$ | 64% | 93 (45:55) : 7 (45:55) |
| 3 N HCl | 67% | 96 (45:55) : 4 (40:60) |
| 3 N HCl (μwave at 140 °C/12 min) | 49% | 89 (53:47) : 11 (49:51) |

**Scheme 8.** Deprotection/epimerization of **25** and **26**.



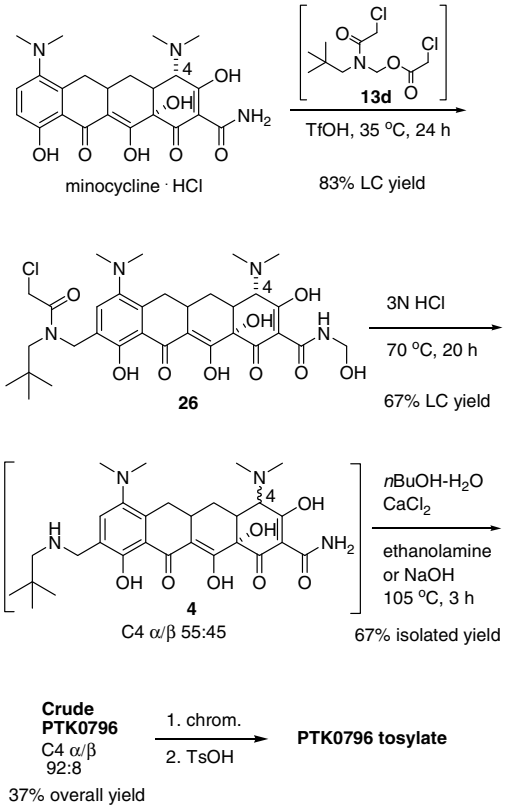

**Scheme 9.** Epimeriation and back-epimerization of PTK0796 C4 center.




**Scheme 10.** One-pot hydrolysis of **26** and back-epimerization of PTK0796 C4 epimer.

PTK0796, an amorphous solid, is unstable at temperatures above 0 °C and when exposed to air. Our salt screening effort found that PTK0796 could be crystallized as a bis-HCl salt, a MSA salt, and a TsOH salt. The crystalline mono-tosylate salt was found to possess significantly improved solid-state stability at 25 °C. This salt also improved the C4 α-epimer to some extent.

In summary, an efficient three-step two-pot convergent synthesis of the aminomethylcycline antibiotic PTK0796 was developed (Scheme 11).[16] Installation of C9 side chain was accomplished in one step via an electronically tuned mono-chloro acyliminium Friedel–Crafts reaction (Tscherniac–Einhorn reaction) with minocycline in 83% yield. Deprotection of the chloroacetyl and hydroxymethyl groups in aqueous HCl, followed by a re-equilibration of the C4 center in a one-pot process afforded crude PTK0796 in 45% yield. A crystalline tosylate salt of PTK0796 was identified as a potential salt for further purification and as a final active pharmaceutical ingredient due to its improved long-term stability.




**Scheme 11.** Three-step two-pot synthesis of PTK0796 via Tscherniac–Einhorn reaction.

## Acknowledgments

We thank Robert Reamer and Peter G. Dormer for their help with NMR.

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7. Iminium and precursors screened included (1) neopentylamine with paraformaldehyde or formalin, (2) *N,N*-bis(methoxymethyl)-2,2-dimethylpropan-1-amine, (3) 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane, (4) *N*-(1*H*-1,2,3-benzotriazol-1-ylmethyl)-2,2-dimethylpropan-1-amine, and (5) *N*-benzyl-*N*-methylenebutan-1-aminium chloride, under acidic, neutral, basic, and Lewis acids in variety of solvents at 0–120 °C.
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16. *Preparation of 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane* (**12**). Neopentylamine (12.2 g, 136.1 mmol) was added to a mixture of paraformaldehyde (4.3 g, 136.1 mmol) in hexanes (85 mL) and heated at reflux (68–71 °C) for 2–3 h. Anhydrous $Na_2SO_4$ (3 g) was added and stirred at 22 °C for 1 h. The mixture was filtered, washed with hexanes (85 mL), and evaporated to dryness to afford 12.78 waxy white solid (94.7%). NMR indicated that the resulting product was ~95 wt % pure. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 3.33 (s, 2H), 2.13

(s, 2H), 0.88 (s, 9H); $^{13}$C NMR ($CDCl_3$, 100.5 MHz) δ 78.6, 64.2, 33.6, 27.8.

*General procedure for the preparation of* **13**. 3.2 equiv of the anhydride and 1 equiv of trizainane **12** were stirred neat or in dichloromethane at the indicated temperature and time. The resulting **13** was used directly in the Friedel–Craft reaction.

*Compound* **13a**: R,R′ = $CF_3$. Trifluoroacetic anhydride (3.2 equiv), 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane (1 equiv), and dichloromethane (1.6 M) were combined and stirred at 25 °C for 20 min. A small aliquot was removed for NMR analysis: $^1$H NMR ($CDCl_3$, 400 MHz) δ 5.70 (s, 2H), 3.42 (s, 2H), 1.00 (s, 9H).

*Compound* **13b**: R,R′ = $CCl_3$. Trichloroacetic anhydride (3.2 equiv), 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane (1 equiv), and dichloromethane (1.6 M) were combined and stirred at 25 °C for 20 min. A small aliquot was removed for NMR analysis: $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.00 (s, 2H), 3.45 (s, 2H), 1.01 (s, 9H).

*Compound* **13c**: R,R′ = $CHCl_2$. Dichloroacetic anhydride (3.2 equiv), 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane (1 equiv), and dichloromethane (1.6 M) were combined and stirred at 40 °C for 30 min. A small aliquot was removed for NMR analysis: $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.49 (s, 1H), 5.98 (s, 1H), 5.67 (s, 2H), 3.40 (s, 2H), 0.96 (s, 9H).

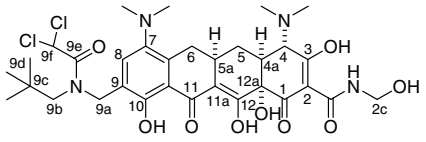
*Compound* **13d**: R,R′ = $CH_2Cl$. Chloroacetic anhydride (3.2 equiv), 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane (1 equiv), and dichloromethane (1.6 M) were combined and stirred at 40 °C for 30 min. A small aliquot was removed for NMR analysis: $^1$H NMR ($CDCl_3$, 400 MHz) δ 5.56 (s, 2H), 4.22 (s, 2H), 4.07 (s, 2H), 3.32 (s, 2H), 0.92 (s, 9H).

*Compound* **13e**: R,R′ = $CH_3$. Acetic anhydride (3.2 equiv) and 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane (1 equiv) were combined and heated at 100 °C for 1 h. A small aliquot was removed for NMR analysis: $^1$H NMR ($CDCl_3$, 400 MHz) δ 5.39 (s, 2H), 3.29 (s, 2H), 2.20 (s, 3H), 2.09 (s, 3H), 0.91 (s, 9H).

*Compound* **13f**: R = *t*-Bu, R′ = H. Formyl pivalic anhydride (3.2 equiv) and 1,3,5-tris(2,2-dimethylpropyl)-1,3,5-triazinane (1 equiv) were combined and heated at 22 °C for 30 min. A small aliquot was removed for NMR analysis. The formamide derivative exists as a 2.5:1 mixture of rotamers: $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.42 (s, 0.7H), 8.15 (s, 0.3H), 5.41 (s, 0.6H), 5.36 (s, 1.4H), 3.20 (s, 1.4H), 3.16 (s, 0.6H), 1.26 (s, 2.6H), 1.24 (s, 6.4H), 0.97 (s, 9H).

*Conversion of minocycline HCl to compound* **25**. Dichloroacetic anhydride (7.78 g, 31.8 mmol) was slowly added to solution of triazinane (3.0 g, 10.0 mmol) and dichloromethane (2 mL) over 20 min with cooling in an ice bath. The solution was stirred at 22 °C for 1 h. In a separate flask, minocycline hydrochloride (3 g, 6.0 mmol) was slowly added to triflic acid (30 mL) with cooling while maintaining at <35 °C. The acyliminium precursor solution was slowly added to minocycline HCl/TfOH solution keeping at <35 °C. The mixture was stirred at 24 °C for 20 h and at 35–40 °C for 4 h, then slowly quenched into 10–15 °C water (120 mL) over 20 min keeping at <25 °C. To the mixture was added dichloromethane (60 mL) and the pH was adjusted to 6.5 with 29% $NH_4OH$ (about 28 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The combined organic layers were extracted with 10 °C 3 N HCl (60 mL). The layers were separated, and the organic was extracted again with 3 N HCl (2 × 20 mL). The combined aqueous phase was maintained at 10 °C and adjusted to pH 6.5 with 29% $NH_4OH$ (about 24 mL). It was warmed to 22 °C, and extracted with dichloromethane (1 × 100 mL, 3 × 20 mL). The combined organic was filtered through $Na_2SO_4$ and the filtrate evaporated to dryness to afford 3.67 g of **25** as an orange solid. The material was estimated to be 77 wt % pure using pure PTK0764 as an HPLC standard. The corrected yield was 76% based on 89% active minocycline HCl.

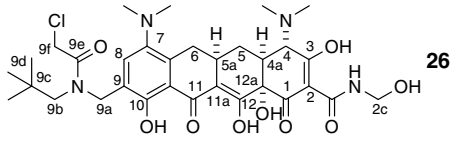
*Compound* **25**. NMR in DMF-$d_9$, indicated that the dichloroacetamide exists as two rotomers: $C_{9f}H$ as singlets at d 7.29 and 7.28; $C_{2c}H_2$ as a triplet at d 6.10; LC/MS *m*/*z* $(M+H)^+$ = 697; HRMS *m*/*z* calcd for $C_{32}H_{43}Cl_2N_4O_9$ $(M+H)^+$ 697.2407, found, 697.2408.




**25**

*Conversion of minocycline HCl to compound* **26**. Chloroacetic anhydride (9.0 g, 53.01 mmol), triazinane (5.0 g, 16.67 mmol), and dichloromethane (10 mL) were combined with cooling in an ice bath. The solution was heated at 35 °C for 20 min, then cooled to 20 °C. In a separate flask, minocycline hydrochloride (5 g, 10 mmol) was slowly added to triflic acid (50 mL) with cooling while maintaining at <35 °C. The acyliminium precursor solution was slowly added to minocycline HCl/TfOH solution keeping at <40 °C. The mixture was heated at 35–40 °C for 24 h, then quenched into 10–15 °C water (200 mL) over 20 min at <25 °C. To this mixture was added dichloromethane (150 mL) and adjusted to pH 6.5 with 29% $NH_4OH$ (about 50 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (3 × 25 mL). The combined organic layers were extracted with 15 °C 3 N HCl (160 mL). The layers were separated and the organic phase was re-extracted with 3 N HCl (1 × 20 mL, 1 × 10 mL). The combined aqueous layers were assayed to contain 73–83% yield of product **26**. At this point, product **26** could be isolated by pH adjustment to 6.5, and extraction with dichloromethane, or taken directly into the next step as described below.

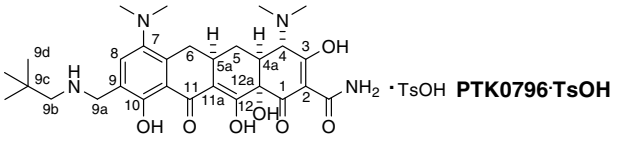
*Compound* **26**. NMR in DMSO-$d_6$ indicated that the chloroacetamide exists as two rotomers: $C_{9f}H_2$ as AB quartets at d 4.40 and 4.35; $C_{2c}H_2$ as a broad singlet at d 4.90; LC/MS *m*/*z* $(M+H)^+$ = 663; HRMS *m*/*z* calcd for $C_{32}H_{44}ClN_4O_9$ $(M+H)^+$ 663.2797, found, 663.2794.




**26**

*Conversion of* **26** *to PTK0769 tosylate*. The aqueous solution from above was heated at 70 °C for 23 h and the resulting solution had a 49% assayed yield of PTK0796 as a ~1:1 mixture of C4 α/β epimers and small amounts of the corresponding *N*-hydroxymethyl analogues. The solution was evaporated to yield 9.7 g of brown oil. *n*-Butanol (50 mL, degassed by $N_2$ bubbling for 5 h, which reduced dissolved oxygen from 8 ppm to 0.07 ppm), degassed water (2.5 mL), $CaCl_2$ (3.92 g), and ethanolamine (4.0 mL) were added. The resulting brown solution with some insoluble $CaCl_2$ was heated to 108 °C for 2 h then cooled to rt. Assay yield was 38% as a 92:8 C4 alpha/beta mixture. To the solution was added Darco KB activated carbon (3 g, 100 mesh) and stirred for 30 min. The solution was filtered through Celite and washed with *n*BuOH (70 mL). The filtrate was diluted with water (60 mL) and adjusted to pH 7.5 with ethanolamine (5 drops). The layers were separated. The organic phase was washed with water (60 mL), then concentrated to dryness. The resulting gummy material was triturated with ethanol and evaporated to dryness to afford 6 g of brown powder with 32 wt % purity (1.92 g or 34% overall yield). The material was purified to >90 wt % purity using reversed phase HPLC method described in International Publication Nos. WO 2005/009944, WO 2004/091513, and WO 2002/004,406. Further purity upgrade to >98% and improved stability at room temperature were achieved via the tosylate salt. The crystalline TsOH salt was prepared by adding a solution of TsOH hydrate (97.0 g) in IPA (400 mL) to a slurry of amorphous PTK0796 free base (289 g) in IPA (2 L) under nitrogen. The water content was adjusted to 0.6 g/L with the addition of water (9 ml) and the slurry was stirred at 20–25 °C for 18 h to produce a thick crystalline slurry. The slurry was filtered and washed with IPA (2 × 500 ml). Excess IPA was removed from the crystalline cake by blowing dry nitrogen through the cake for 24 h. With the solids containing 3 wt % IPA, the cake was further dried by blowing humidified nitrogen through the cake at a relative humidity of 70–75% for 24 hr. The cake retained 0.9 wt % IPA that was not further reduced by this method. Excess water was then removed from the cake by blowing dry nitrogen through the cake for 24 h to afford 337 g of PTK0796 TsOH salt with >98% purity.

*PTK0796 tosylate*: $^1$H NMR ($D_2O$, 500 MHz) δ 7.63 (d, *J* = 8.1, $C_{2'}H$, $C_{6'}H$), 7.52 (s, $C_8$ H), 7.28 (d, *J* = 8.1, $C_{3'}H$, $C_{5'}H$), 4.30 (s, $C_{9a}H_2$), 3.80 (d, *J* = 1.1, $C_4H$), 3.11 (dd, *J* = 15.6, 4.2, $C_6H$), 2.95 (s, 4-N$(CH_3)_2$), 2.89 (m, $C_{5a}$ H), 2.84 (s, $C_{9b}H_2$), 2.72 (dt, *J* = 12.7, 2.2, $C_{4a}H$), 2.62 (s, 7-N$(CH_3)_2$), 2.34 (s, $C_{4a'}H_3$), 2.24–2.18 (m, $C_5H$, $C_6H$), 1.66 (td, *J* = 13.5, 11.5, $C_5H$), 1.01 (s, $C_{9d}H_9$); PTK0796 C4 epimer has resonances at δ 4.58 (d, *J* = 3.6), 2.99 (s), 2.59 (s); $^{13}$C NMR ($D_2O$, 125.7 MHz) δ193.77 ($C_{11}$), 191.90 ($C_1$), 184.86 ($C_3$), 174.23 ($C_{12}$), 171.59 ($C_{2a}$), 156.94 ($C_{10}$), 142.84 ($C_{4'}$), 142.31 ($C_7$), 140.30 ($C_{1'}$), 139.17 ($C_{6a}$), 130.18 ($C_8$), 129.98 ($C_{2'}$, $C_{6'}$), 125.96 ($C_{3'}$, $C_{5'}$), 117.59 ($C_{10a}$), 117.26 ($C_9$), 109.20 ($C_{11a}$), 102.82 ($C_2$), 75.97 ($C_{12a}$), 71.69 ($C_4$), 58.59 ($C_{9b}$), 47.84 ($C_{9a}$), 45.08 (7-N$(CH_3)_2$), 41.59 (br s, 4-N$(CH_3)_2$), 34.70 ($C_5$), 34.08 ($C_{4a}$), 32.48 ($C_{5a}$), 30.73 ($C_6$), 30.33 ($C_{9c}$), 26.83 ($C_{9d}$), 21.15 ($C_{4a'}$); LC/MS, *m*/*z* $(M+H)^+$ = 557.




·TsOH **PTK0796·TsOH**

*Alternate back-epimerization procedure using NaOH*. Friedel–Crafts product **26** (~70 mg, ~0.1 mmol) and $CaCl_2$ (40 mg) were dissolved in 3 N HCl (1 mL). The solution was heated to 70 °C for 14 h (or until starting material disappears). 5 N NaOH (0.76 mL, 3.8 mmol) and 1 mL *n*BuOH (two layers) were added. The solution was heated at 105–110 °C for 4–5 h, then worked up as described above.