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A highly diastereoselective vinylogous Mannich condensation and 1,4-conjugate addition of (*Z*)-propenyl cuprate in the synthesis of an influenza neuraminidase inhibitor

David M. Barnes, Lakshmi Bhagavatula, John DeMattei,[†] Ashok Gupta, David R. Hill, Sukumar Manna, Maureen A. McLaughlin, Paul Nichols,[†] Ramiya Premchandran,[‡] Michael W. Rasmussen, Zhenping Tian and Steven J. Wittenberger*

Abbott Laboratories, Global Pharmaceutical Research and Development, Process Research and Development, 1401 Sheridan Road, North Chicago, IL 60064-4000, USA

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Abstract—A practical synthesis of neuraminidase influenza inhibitor, A-322278, has been developed. Asymmetry is introduced into the synthesis by an enzyme mediated ester hydrolysis. A highly diastereoselective vinylogous Mannich condensation reaction of *N*-Boc-2-*tert*-butyldimethylsilyloxypyrrole (TBSOP) and an *N*-(triphenylmethylsulfenyl)imine proceeds under thermodynamic control to assemble the framework. A significant temperature dependent rate difference for the transfer of (*Z*)- and (*E*)-propenyl moieties from a cuprate reagent during a 1,4-conjugate addition was observed. A very selective addition of cyanide to an *N*-acyliminium intermediate was employed to control the final stereocenter.

1. Introduction

Influenza (the 'flu') is a respiratory tract infection caused by the influenza virus. Between 25 and 75 million people in the United States are infected annually and cost estimates for heath care and lost productivity are in excess of 10 billion dollars per year.[1] Although most people recover in 1 to 2 weeks, this illness can be quite serious. In the United States, influenza is associated with more than 20,000 deaths and more than 100,000 hospitalizations in an average year.[2] At the present time, there are four approved medications available in the US for the control and prevention of influenza: amantadine, rimantadine, zanamivir, and oseltamivir. Amantadine and rimantadine are active only against influenza type A viruses. Zanamivir and oseltamivir are influenza neuraminidase inhibitors (NI) with activity against both of the clinically important influenza viruses, A and B. Inhibition of neuraminidase impairs the ability of the newly produced virus to be released from the infected cell, thereby disrupting viral replication. All of these agents can reduce the severity and duration of uncomplicated influenza infection by viral strains against which they are active.[3]

Abbott Laboratories' influenza neuraminidase inhibitor, A-315675 **1**, is a potent inhibitor of influenza A and B viral replication.[4] The initial synthetic route provided this material in racemic form; gram quantities of compound for early biological evaluation were resolved by preparative HPLC on a chiral stationary support. As greater quantities of material would be required for development, it was obvious that a more efficient, asymmetric synthesis was necessary. Such an approach was demonstrated that allowed for multigram quantities of drug to be prepared.[5] Our studies targeted several key stereochemical and practical issues left unresolved from this initial work. Herein the process research and initial scale up to prepare bulk quantities of A-322278 **2**, a pro-drug of A-315675, required for further pre-clinical evaluation is described (Fig. 1).[6]

2. Results and discussion

The starting point for our research was the asymmetric route previously demonstrated by our colleagues.[5] This approach is shown retrosynthetically in Scheme 1 to highlight the key transformations. The C-2 carboxylate

* Corresponding author. E-mail: steve.j.wittenberger@abbott.com
[†] Present address: Array Biopharma, 3200 Walnut Street, Boulder, CO 80301.
[‡] Present address: MediChem Life Sciences, Woodridge, IL 60517.

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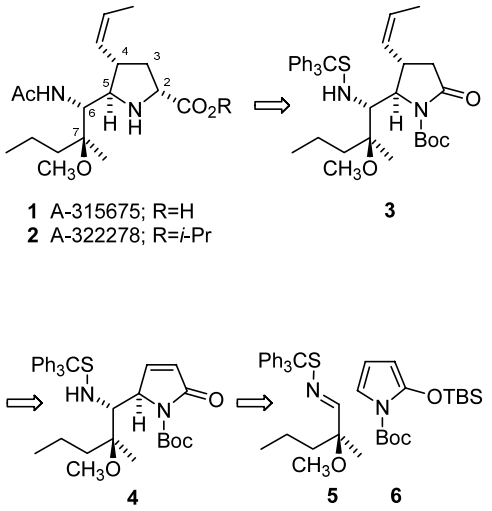
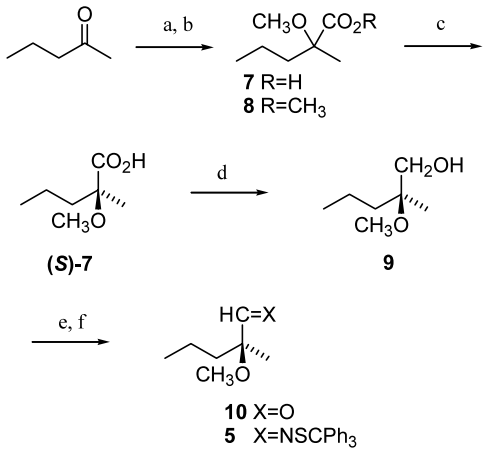


Figure 1.

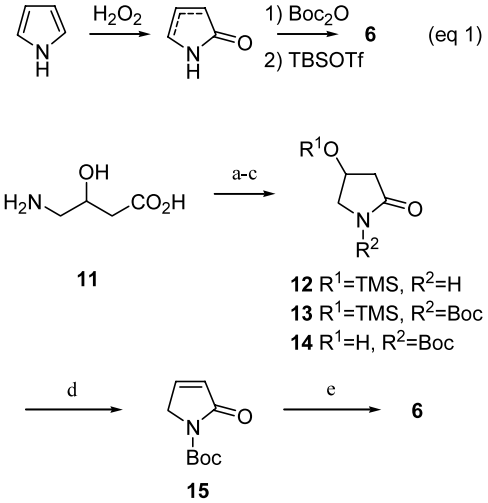


Scheme 1. *Reagents and conditions*: (a) $CHBr_3$, KOH, MeOH; (b) MeOH, H_2SO_4 (cat.); (c) lipase *Candida rugosa*, NaOH (aq), acetone; (d) BH_3-SMe_2; (e) DMSO, SO_3–pyridine complex, Et_3N; (f) H_2NSCPh_3, Na_2SO_4.

of **1** (or **2**) was derived from the corresponding C-2 nitrile which was prepared by a stereoselective addition of cyanide to the *N*-acyliminium species generated from reduction of *N*-Boc lactam **3** followed by activation with a Lewis acid. The (*Z*)-propenyl side chain at C-4 was incorporated via conjugate addition of a cuprate reagent to *N*-Boc-Δ^3-2-pyrrolidinone **4**. In the pivotal reaction of this sequence, **4** was formed by the stereoselective condensation of imine **5** and *N*-Boc-2-(*tert*-butyldimethylsilyloxy)pyrrole (TBSOP) **6**. This reaction is an extension of the studies of Casiraghi et al. on the Lewis acid promoted condensations of pyrrole-, furan-, and thiophene-based 2-siloxydienes with aldehydes and, to a lesser extent, imines.[7] Thus, efficient preparations of these two starting materials, imine **5** and TBSOP **6**, became early goals.

Early supplies were prepared through the use of the Sharpless asymmetric epoxidation reaction, however, throughput was low and an alternate method was developed to prepare **5**. Reaction of 2-pentanone with bromoform in methanolic potassium hydroxide solution provided carboxylic acid **7** in 80% yield.[8] Attempts to accomplish an efficient classical resolution of (±)-**7** with commercially available enantiomerically pure amines failed. Therefore, a series of simple carboxylic esters were prepared and screened versus a battery of readily available enzymes in an effort to achieve resolution by kinetic hydrolysis. The combination of methyl ester (±)-**8** and lipase from *Candida rugosa* proved to be very effective; 49% yield (98% of a theoretical 50%) of $\geq$97%ee carboxylic acid (*S*)-**7** was routinely obtained on multi-hundred gram scale. Reduction of acid (*S*)-**7** with BH_3·SMe_2 provided alcohol **9** in near quantitative yield. Oxidation followed by condensation with triphenylmethanesulfenamide gave **5** in 89% yield.

The method[9] reported by Casiraghi for the preparation of *N*-Boc-2-(*tert*-butyldimethylsiloxy)pyrrole **6** (TBSOP) was unsuitable for large-scale use (Scheme 2, Eq. (1)). The oxidation of pyrrole with hydrogen peroxide posed safety concerns if carried out on large scale and the purification was tedious.[10] An alternate synthesis was developed in order to safely and reliably produce multi-kilogram quantities of **6**. We were prompted by a paper from the Pifferi group to explore the dehydrative cyclization of (±)-4-amino-3-hydroxybutyric acid **11** employing HMDS and TMSCl in refluxing xylene.[11] In our laboratory the product yield was quite variable (60–95%) with seemingly uncontrollable amounts of des-silyl by-product as the remainder; however, exposure of **11** to HMDS and pyridine in refluxing xylene led to a high yield of **12** (91–98%). Concentration of the solvent removed the volatile reaction by-products. Crude **12** was dissolved in THF and treated initially with di-*tert*-butyl dicarbonate and DMAP to introduce the Boc group and provide (±)-*N*-



Scheme 2. *Reagents and conditions*: (a) HMDS, C_5H_5N, xylene, Δ; (b) Boc_2O, DMAP; (c) Et_3N-3HF; (d) MsCl, Et_3N; (e) TBSOTf, Et_3N.

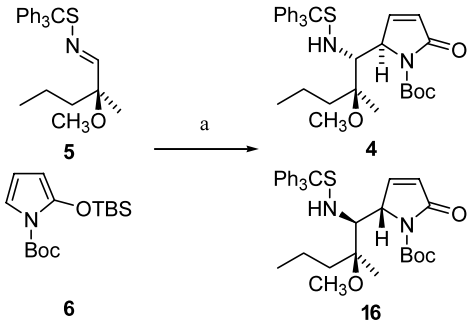
Boc-4-(trimethylsiloxy)-2-pyrrolidinone **13**. Upon reaction completion, triethylamine trihydrofluoride ($Et_3N{\cdot}3HF$) was added to the mixture to remove the trimethylsilyl moiety. As the deprotection proceeded, the product (±)-*N*-Boc-4-hydroxy-2-pyrrolidinone **14** crystallized from the reaction mixture. This one-pot three-step sequence provided **14** in 97% overall yield in the laboratory and 89% yield on a 15 kg scale in the pilot plant.[12]

The stable crystalline **14** was a convenient intermediate to stockpile for ready access to TBSOP. Dehydration was carried out by treatment with methanesulfonyl chloride and triethylamine to give the *N*-Boc-Δ^3-pyrrolidinone **15**. To a mixture of crude **15** and heptane was added triethylamine and diatomaceous earth. After cooling to 0°C the mixture was treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf). The triethylammonium trifluoromethanesulfonate formed as a by-product was absorbed onto the filter agent as the reaction progressed. At the end of reaction, the darkened diatomaceous earth was removed by filtration. All other reaction by-products were volatile and were removed upon concentration of the filtrate. For use in our process, the residue was reconstituted in heptane and held for next step; the yield of TBSOP **6** was 95–98% (85–95% overall).

From the outset, it was very apparent that achieving an efficient and selective coupling of imine **5** and TBSOP **6** was key to the success of this strategy. Our colleagues had found that reaction of **5** and **6** in the presence of $BF_3{\cdot}Et_2O$ resulted in the formation of two products, **4** and **16**, in a 3–7:1 ratio (75% combined yield) along with some unreacted starting materials.[5] Screening of Lewis acids and solvents resulted in modest improvement in diastereoselectivity with TMSOTf in THF at −78°C (6:1, **4**:**16**).

During optimization experiments, two important observations were made. First, the ratio of product diastereomers was observed to improve during the course of some reactions. By extending reaction times (15 h versus 3–4 h) or increasing the reaction temperature (to −40°C) the product diastereomeric ratio improved markedly (11:1, 83% assay yield and 19:1, 95%, respectively). Re-subjecting purified **4** and **16** to the reaction conditions provided additional evidence for equilibration of the products under the reaction conditions. In the case of **4**, the ratio (**4**:**16**) changed from >99:1 to 93:7, which was consistent with previous experiments. Exposure of **16** (2:98) led to a ratio of 26:74. In both experiments there was substantial degradation of the products due to loss of the *N*-Boc group which may explain why equilibrium ratios were not met (especially in the latter case) (Scheme 3).

The second observation was that reactions on small scale (50–100 mg) proceeded to completion and provided high yields of product. Upon increasing to gram scale however, reactions would tend to stall and could not be pushed to completion even with additional aliquots of starting materials. This led to the idea that

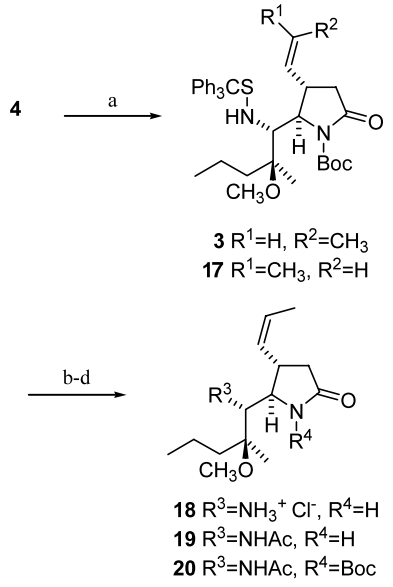


Scheme 3. *Reagents and conditions*: (a) Optimized conditions: TfOH, THF, −40°C.

the presence of small amounts of residual water might be responsible for the better results on small scale. Replacing TMSOTf with trifluoromethanesulfonic acid resulted in a robust and reproducible procedure where **5** and **6** reacted to produce a 19:1 ratio of **4** and **16** in 90–95% HPLC assay yield. Following workup, the major product **4** was isolated by crystallization in 80–85% yield in >99:1 diastereomeric purity. Model studies and calculations provided evidence to understand the origins of the high selectivity observed; under the reaction conditions an intramolecular hydrogen bond between the C-6 *S*-triphenylmethyl ammonium substituent and the C-7 methoxy group stabilizes the major diastereomer **4** relative to the minor diastereomer **16**.[13]

At this stage, a number of preliminary investigations of strategies to incorporate the (*Z*)-1-propenyl moiety were conducted. Our endeavors quickly focused upon the use of $[(Z)\text{-1-propenyl}]_2CuMgBr$ derived from the corresponding Grignard reagent and $CuBr{\cdot}SMe_2$ complex due to the high isomeric purity of commercially available (*Z*)-1-bromo-1-propene[14] and the high yield of the 1,4-conjugate addition products (Scheme 4). Early experiments showed that removing the (*E*)-1-propenyl adduct **17** from the desired (*Z*)-isomer **3** would be extremely difficult either by crystallization or chromatography after the 1,4-conjugate addition or later in the synthesis, so a goal was established to develop conditions to control this impurity to <1% in the cuprate addition reaction.

(*Z*)-1-Bromo-1-propene is sensitive to thermal and acid-catalyzed isomerization.[15] In contrast to earlier reports[15,16] however, we observed complete retention of configuration on preparation of the Grignard reagent in THF.[17] A brief survey of copper(I) salts showed that $CuBr{\cdot}SMe_2$, CuI, or CuCN could be used to prepare cuprate reagents that produced the 1,4-conjugate addition products in high yield. The mixed higher order cuprate reagent[18] derived from CuCN, 2-thienyllithium and (*Z*)-1-propenylmagnesium bromide also yielded the desired product, however, it was contaminated by several low level impurities that were not observed with the other reagents. $CuBr{\cdot}SMe_2$ complex was chosen for scale up because of its relative ease in handling and reduced toxicological liability relative to copper(I) cyanide.

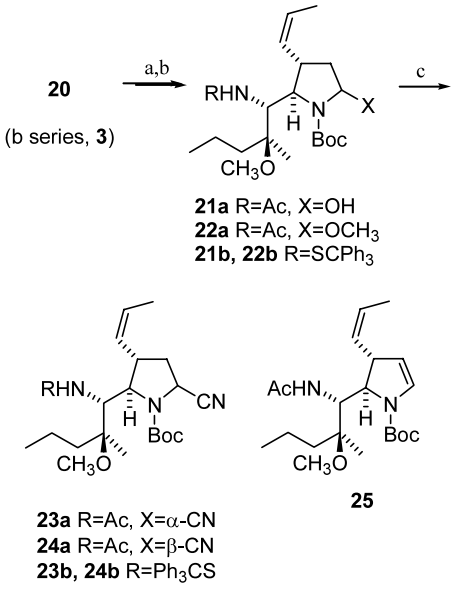


Scheme 4. *Reagents and conditions*: (a) (*E*)-propenyl cuprate, TMSCl, THF/toluene, −20°C; (b) HCl (g); (c) NaOH (aq), Ac_2O; (d) Boc_2O, DMAP.

Early in the development of this reaction, the cuprate reagent was prepared in excess from $CuBr{\cdot}SMe_2$ (5 equiv.) and (*Z*)-$CH_3CH{=}CHMgBr$ (10 equiv.) in THF at −78°C. Chlorotrimethylsilane (3 equiv.) was added, followed by a THF solution of **4**. Typically a high yield (≥95%) of desired adduct **3** was obtained along with 2–4% of isomer **17**.[5,19] The quantities of reagents could be reduced ($CuBr{\cdot}SMe_2$ (0.5 equiv.) and (*Z*)-$CH_3CH{=}CHMgBr$ (3 equiv.)) without effect; lower amounts gave incomplete conversion. We investigated alternate modes of addition in an effort to conserve (*Z*)-1-bromo-1-propene because it was an expensive reagent. By adding the Grignard reagent to a mixture of **4**, $CuBr{\cdot}SMe_2$, and TMSCl only 2.1 equiv. (*Z*)-$CH_3CH{=}CHMgBr$ was required to consume **4**, however, the level of *trans*-product **17** was high (≥3%).

We were pleasantly surprised on investigating the effect of reaction temperature. With our preferred stoichiometries for the pre-formed cuprate reagent[20] (0.5 equiv. $CuBr{\cdot}SMe_2$, 3 equiv. (*Z*)-$CH_3CH{=}CHMgBr$, and 1.2 equiv. TMSCl in THF) at −78°C, 1.6% of *trans*-product **17** was observed, but at −20 to −25°C only 0.5–0.7% **17** was detected! It is important to note that this was the last point in the synthetic scheme that a sensitive analytical method was in place for determination of the (*Z*):(*E*)-olefin ratio prior to final product. The selective transfer of the (*Z*)-1-propenyl moiety from $[(Z)\text{-1-propenyl}]_2CuMgBr$ in a 1,4-conjugate addition reaction has been previously observed.[21] Considering that the Grignard reagent (and the derived cuprate reagent, measured by assay of vinyl iodides following iodine quench) contained 3% (*E*)-1-propenyl ligand, the rate difference for the transfer of the (*Z*)-1-propenyl unit versus the (*E*)-1-propenyl unit was judged to be 2–4:1 at −78°C but increases to 6–12:1 at −20 to −25°C.[22] Reactions that were run at temperatures higher than −20°C required additional Grignard reagent to go to completion and provided lower product yield with increased levels of *trans*-isomer **17**.

Conjugate addition product **3** was carried on without purification since (a) the crude product was devoid of any significant impurities and (b) there were concerns about the stability of the triphenylmethylsulfenamine moiety.[23] Investigations into the selective removal of the triphenylmethylsulfenyl group[24] were not promising; under a variety of conditions, cleavage of the *N*-Boc group was competitive. Selectivity could be achieved, but the required conditions would be challenging to control on large-scale (i.e. iodotrimethylsilane at −78°C) or difficult to purify from the triphenylmethylsulfenyl by-products (i.e. zinc in acetic acid). The most pragmatic solution was a global deprotection–reprotection sequence (Scheme 4). Exposure of the organic phase following work up of the cuprate reaction to gaseous HCl resulted in rapid and complete removal of both the triphenylmethylsulfenyl and *N*-Boc groups. Addition of water partitioned ammonium salt **18** into the aqueous phase and the triphenylmethylsulfenyl by-products remained in the organic layer. Following pH adjustment, the solution was treated with acetic anhydride to provide acetamide **19**. This compound was sufficiently hydrophobic to be extracted into isopropyl acetate after salting the aqueous phase. After azetropic drying and switching the solvent to THF, **19** was treated with di-*tert*-butyl dicarbonate, triethylamine and catalytic DMAP. The product imide **20** was isolated by crystallization in 87% yield over the four steps from **4** (Scheme 5).



Scheme 5. *Reagents and conditions*: (a) $LiB(Et_3)H$, THF; (b) $CH_3C(OCH_3)_3$, CSA; (c) TMSCN, TfOH, CH_3CN, −42°C.

Imide reduction of **20** could be accomplished with either DIBAL-H or lithium triethylborohydride to produce **21a** as a mixture of diastereomers. $LiB(Et)_3H$ was selected because the crude reaction mixture appeared slightly cleaner and the work up was simpler due to the absence of aluminum salts. Exposure of crude **21a** to trimethyl orthoacetate and methanol with catalytic camphorsulfonic acid yielded *N*-Boc-methoxyaminal **22a** in 96% yield from imide **20**. As noted above, a sensitive assay to measure the (*Z*):(*E*)-olefin ratio had not been developed between cuprate adduct **3** and final product **2**. After completing our initial scale up campaign, it was discovered that some isomerization (0.7% (*E*)→1.7% (*E*)) of the (*Z*)-propenyl moiety had occurred. A thorough investigation of intermediate samples and reaction conditions determined that the loss of stereochemistry had occurred in the $LiB(Et)_3H$ reduction step! With this finding, we suspected that $B(Et)_3$ in the hydride reagent might be responsible as it is well known that trialkylboranes and oxygen are a source of radical species.[25] In fact, in parallel reactions **20** (0.5% *trans*-olefin isomer) was treated with $B(Et)_3$ (0.26 equiv.) in the presence and absence of oxygen under conditions similar to the reduction reaction (0.13 M in THF, −10 to −15°C). With oxygen, 2.3% (*E*)-propenyl isomer was detected, while the control reaction showed 0.5% (*E*)-isomer.[26]

Incorporation of the C2-carboxylate established the final stereogenic center. Nucleophilic addition of cyanide anion to the *N*-acyliminium ion (Fig. 2) generated from **22a** or **22b** was expected to proceed anti to the bulky C5-substituent that was envisioned to adopt a pseudo-axial conformation to minimize $A^{1,2}$ strain with the *N*-Boc group.[27] Early studies conducted on the triphenylmethylsulfenyl protected derivative **22b** showed that the choice of Lewis acid and solvent[28] had a significant impact the reaction yield and selectivity (Table 1, entries 1–7). This proclivity was encountered again with **22a**, which was pursued for development after encountering stability problems with the triphenylmethylsulfenyl moiety.[23] Nevertheless, the optimized conditions for **22a** (3.0 equiv. TMSCN, 1.5 equiv. TfOH, CH_3CN, −40°C) were reproducible in providing 2-cyanopyrrolidine adducts **23a** and **24a** in 85–90% yield in a 96:4 diastereomeric ratio. Approximately 5–8% yield of the des-Boc product was also typically observed.[29] Results with the 2-ethoxy analogue of **22a** were similar (**23a** in 80% yield), but the 2-isopropoxy ether and *N*-Boc-hydroxyaminal **21a** were inferior (18 and 48%, respectively) with significant amounts of the elimination side product **25** formed. In practice the crude **23a** containing ~4% epimeric **24a** was carried into the final reaction sequence.

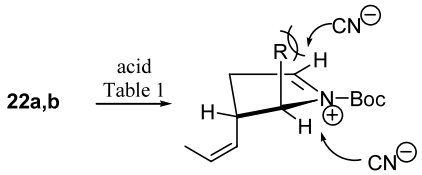


Figure 2.

The transformation of **23a** to the target A-322278 **2** could be accomplished in three steps through the intermediacy of the amino acid **1** by exposure to 6N HCl (0.8 mM, 60°C, 16 h, 100% yield)[5,6] followed by treatment with thionyl chloride and *i*-PrOH (rt, 48 h, **2**, 31% yield, unoptimized) and tosylate salt formation.[30] From a processing perspective, however, a more direct conversion that avoided chromatographic purification was desired. After several iterations of optimization studies, during which time our analytical methods steadily improved, we came to the following conclusions. First, the hydrolysis/alcoholysis of the nitrile would have to be carried out under acidic conditions as exposure to base caused rapid epimerization of the nitrile to the thermodynamically favored β-isomer. Second, HCl was the acid of choice as a host of others resulted in significant amounts of diketopiperazine **26** (Fig. 3). The empirical observations were that build up of the amino nitrile intermediate **27** correlated with formation of **26** and that HCl facilitated rapid alcoholysis at low temperature. Finally, incorporation of HCl into the molecule,[31] presumably by addition across the alkene,

Table 1. Optimization of nitrile addition

Entry	Substrate	Lewis acid	Solvent	Temp. (°C)	% Yield **30**	α:β ratio
1	**22b**	$BF_3{\cdot}OEt_2$	CH_2Cl_2	−78	89	~11:1
2	**22b**	$BF_3{\cdot}OEt_2$	CH_3CN	−30	Nd	~1:1
3	**22b**	$BF_3{\cdot}OEt_2$	THF	−78	Nd	~10:1
4	**22b**	$BF_3{\cdot}OEt_2$	Toluene	−78	Nd	~1:1
5	**22b**	$SnCl_4$	Toluene	−78 to −45	32	1.5:1
6	**22b**	TMSOTf	CH_2Cl_2	−78	Nd	6:1
7	**22b**	TMSOTf	Toluene	−78	68	~3:1
8	**22a**	$BF_3{\cdot}OEt_2$	CH_2Cl_2	−78	Nd	~2:1
9	**22a**	$BF_3{\cdot}OEt_2$	Toluene	−78	Nd	~1:1
10	**22a**	$BF_3{\cdot}OEt_2$	THF	−78	NR	–
11	**22a**	TMSOTf	CH_3CN	−42	67	93:7
12	**22a**	TfOH	CH_2Cl_2	−42	50	90:10
13	**22a**	TfOH	Toluene	−42	60	88:12
14	**22a**	TfOH	THF	−42	19	87:13
15	**22a**	TfOH	CH_3CN	−42	90	96:4

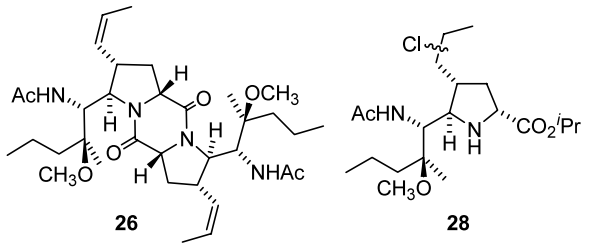
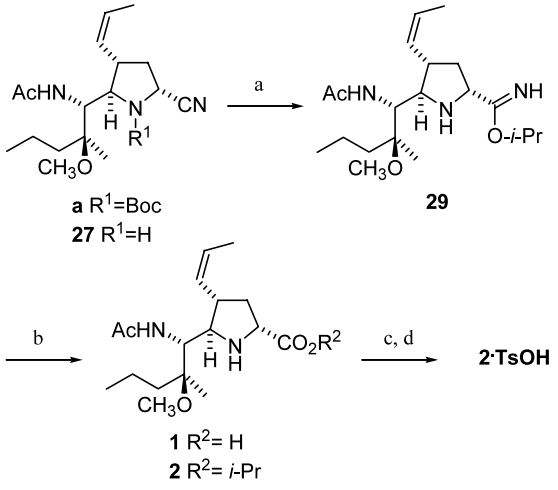


Figure 3.

could occur at and above ambient temperature to give **28** (Fig. 3, up to several percent in some cases) under the very acidic conditions necessary to efficiently convert **23a** into **2**, via imidate **29**.

The optimized procedure for this Pinner reaction entailed treatment of a cold solution of **23a** in *i*-PrOH with anhydrous HCl (Scheme 6). Cleavage of the *N*-Boc group was very fast. Upon warming to about rt, HPLC analysis of the reaction mixture showed that **27** was consumed. The reaction mixture was diluted with *i*-PrOH. In effect this reduced the HCl concentration and minimized formation of **28**. The reaction mixture was warmed to reflux for several hours to convert the intermediate imidate **29** to the isopropyl ester **2**. There was some erosion in the C-2 α:β diastereomer ratio, 96:4 in **23a** to 92:8 in **2**. Typically a small amount of the amino acid **1** was produced in the reaction and it was not converted to **2** under these conditions. After cooling to rt, the reaction mixture was concentrated to half-volume to remove most of the HCl, diluted with isopropyl acetate (*i*-PrOAc), and washed with 15% wt aqueous potassium bicarbonate solution. This wash removed the small amount of amino acid **1** and provided an organic solution of **2** free base. Following a brine wash, the wet organic solution of **2** (ca. 2–3% wt water by KF titration) was azeotropically dried and filtered to remove precipitated inorganic salts that deposited. Addition of an *i*-PrOAc solution of *para*-toluenesulfonic acid monohydrate resulted in the precipitation of **2**·TsOH, which was isolated in 84% yield.



Scheme 6. *Reagents and conditions*: (a) HCl (g), *i*-PrOH, −30°C to rt; (b) add *i*-PrOH, Δ; (c) $KHCO_3$ (aq), *i*-PrOAc; (d) $TsOH{\cdot}H_2O$, *i*-PrOAc.

3. Conclusions

In the course of developing a practical synthesis of influenza neuraminidase inhibitor A-322278, a number of interesting observations were made and several synthetic challenges were overcome. A new preparation of *N*-Boc-2-*tert*-butyldimethylsilyloxypyrrole (TBSOP) **6** that is amendable to large-scale was demonstrated.[12] A short route to (2*S*)-2-methoxy-2-methyl-pentylidene-(triphenyl-methylsulfenyl)amine **5** that featured an extremely efficient enzymatic resolution established asymmetry in the synthesis. These subunits were coupled in a remarkably diastereoselective vinylogous Mannich reaction under thermodynamic control.[13] The (*Z*)-1-propenyl substituent was introduced by a 1,4-conjugate addition reaction to **4**. A temperature dependent kinetic preference for transfer of the (*Z*)-isomer relative to the (*E*)-1-propenyl isomer from the organocopper reagent was observed and the relative rate difference was calculated. We unexpectedly experienced ~1% erosion in alkene geometry of the (*Z*)-1-propenyl substituent due to presence of triethylborane and oxygen during the lithium triethylborohydride reduction of an imide. The C-2 carboxylate functionality was incorporated by the nucleophilic addition of cyanide ion to an *N*-acyliminium ion with a high level of stereochemical control. The end game of *N*-Boc deprotection and nitrile alcoholysis was carried out in one pot under conditions that minimized the formation of diketopiperazine and HCl olefin addition side products. This synthetic route, which proceeds in 16 steps by the longest linear sequence (including an enzymatic resolution) and 12.9% overall yield, was used to successfully deliver more than 1 kg A-322278·TsOH to support pre-clinical biological, toxicological and drug safety evaluations.

4. Experimental

4.1. General procedures

Starting materials, reagents, and solvents were purchased from commercial suppliers and were used without further purification. ^1H NMR spectra were recorded at 300, 400 or 500 MHz and ^{13}C NMR spectra were recorded at 75 or 100 MHz with chemical shifts (δ ppm) reported relative to tetramethylsilane (TMS) as an internal standard. Melting points were determined on a Thomas–Hoover capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Robertson Microlit Laboratories. Column chromatography was carried out on silica gel 60 (230–400 mesh). Thin-layer chromatography (TLC) was performed using 250 mm silica gel 60 glass-backed plates with F254 as indicator. Visualization of TLC was carried out by UV light, $KMnO_4$ or phosphomolybdic acid spray reagent.

4.2. (±)-Methyl-2-methoxy-2-methyl-pentanoate, 8

A solution of (±)-**7**[8] (2.5 kg, 17.1 mol) in methanol (12 L) was treated with concentrated H_2SO_4 (200 mL) and heated to reflux for 16 h. After cooling to ambient temperature, the reaction mixture was treated with $NaHCO_3$ (1.5 kg) and concentrated. The residue was dissolved in MTBE (8 L) and extracted with 0.5%wt $NaHCO_3$ solution (4 L). The organic layer was concentrated and the residue distilled at 50 mmHg; the fraction boiling at 90–95°C was collected to provide **8** (2.1 kg, 77% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.7 (s, 3H), 3.25 (s, 3H), 1.7–1.6 (m, 2H), 1.35 (3, 3H), 1.3–1.15 (m, 2H), 0.89 (t, $J=7.0$ Hz, 3H).

4.3. (2*S*)-2-Methoxy-2-methyl-pentanoic acid, (*S*)-7

A reactor equipped with a mechanical stirrer and temperature probe was charged with *Candida rugosa* lipase (800 g, 875 units/mg), 0.05 M phosphate buffer (pH 7, 16 L), **8** (0.8 kg, 5 mol) and acetone (1.6 L). The mixture was warmed to 35–37°C for ~120 h with pH adjustment to 6 with 50% NaOH every 24 h. Upon completion, the conversion was 51.2% and (*S*)-**7** formed was 97.8% ee. The pH of the reaction mixture was adjusted to 3 with concentrated HCl and extracted with EtOAc (20 L). The organic layer was extracted with 10% wt Na_2CO_3 solution (2×5 L). The combined aqueous portions were acidified with concentrated HCl to pH 3 and were extracted with MTBE (24 L). The organic phase was dried over Na_2SO_4, filtered and concentrated to provide (*S*)-**7** (339 g, 46% yield, 97% ee). Chiral GC analysis conditions: J&W CyclodexB column, 30 m×0.32 mm id, 0.25 μm df; He at 2.5 mL/min (~44 cm/s); oven temperature: 100°C for 14 min then 10°C/min to 120°C then hold 5 min; 1 μL injection at 2–3 mg/mL, 50:1 split, 250°C; FID detection, 300°C w/He at 30 mL/min; enantiomer peaks at 11.4 min (desired) and 11.8 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.6 (s, 1H), 3.26 (s, 3H), 1.8–1.23 (m, 4H), 1.39 (s, 3H), 0.89 (t, $J=7.0$ Hz, 3H).

4.4. (2*S*)-2-Methoxy-2-methyl-hexan-1-ol, 9

A solution of (*S*)-**7** (550 g, 3.77 mole) in CH_2Cl_2 (2.75 L) at 0°C was treated with 10 M BH_3-SMe_2 (0.68 L, 1.8 equiv.). The mixture was stirred 5 h at 20–25°C then 2 M NaOH (3.02 L) was added. The layers were separated; the aqueous layer was extracted with CH_2Cl_2. The organic layer was assayed by GC for **9** (423 g, 85% yield). GC analysis conditions: HP-5 (crosslinked 5% PH ME Siloxane, 30 m×0.32 mm×0.25 mm); column pressure 25 psi; oven temperature: 29°C 2 min, increase 10°C/min to 100°C then hold 2 min; 1 μL injection at 2–3 mg/mL, 100:1 split, 250°C; FID detection, 300°C w/He at 7.7 mL/min; retention time is 4.9 min.

4.5. (2*S*)-2-Methoxy-2-methyl-pentylidene-*N*-triphenylmethylsulfenamine, 5

To a solution of alcohol **9** (19.21 g, 145 mmol) and Et_3N (43.5 g, 430 mmol) in CH_2Cl_2 (100 mL) chilled to ≤5°C was added a solution of SO_3·pyridine complex (35.0 g, 220 mmol) in DMSO (160 mL) over ca. 2.5 h to maintain the reaction temperature <10°C. Following the addition, the reaction was monitored by GC. The reaction was quenched by the addition of 2 M H_3PO_4 (325 mL) at a rate to maintain internal temperature <20°C. The layers were separated and the upper aqueous phase was extracted with CH_2Cl_2 (160 mL). The combined organics were washed with 2 M H_3PO_4 (160 mL) and then dried over silica gel (9.5 g). Filtration and careful partial solvent distillation gave 60.4 g of a solution that contained **10** (17.97 g, 137.6 mmol, 95.3% yield) by GC assay: DB-1 column, 30 m×1.5 μm film×0.53 mm; temperature gradient: 40°C for 3 min then ramp to 190°C at 15°C/min, hold at 190°C 3 min (16 min run time); FID detection, **9** (8.6 min), aldehyde **10** (7.5 min). **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58 (s, 1H), 3.28 (s, 3H), 1.69–1.51 (m, 2H), 1.45–1.25 (m, 2H), 1.22 (s, 3H), 0.92 (t, $J=7.0$ Hz, 3H). To this solution was added Ph_3CSNH_2 (39.30 g, 134.9 mmol), PPTS (0.69 g, 2.7 mmol), and Na_2SO_4 (9.81 g, 69 mmol); the mixture was stirred at rt ca. 12 h. The disappearance of **10** was monitored by GC. After the reaction was judged to be complete, the mixture was diluted with heptane (32 mL) and the solid Na_2SO_4 was filtered, rinsing with heptane. The solution was concentrated at reduced pressure to remove residual CH_2Cl_2 and obtain a ~50% wt solution of imine **5**. The solution was filtered through a pad of silica gel (64 g); the pad was rinsed with 5% MTBE/heptane (500 mL). The filtrate was concentrated in vacuo to a 40–50% wt solution that contained imine **5** (50.6 g, 125 mmol, 93.0% yield) by HPLC assay: Zorbax SB-C8 250 mm×4.6 mm column, gradient 50% CH_3CN/0.1% H_3PO_4 aq to 90% CH_3CN/0.1% H_3PO_4 aq over 15 min, 1.5 mL/min, column temp. 35°C, UV at 210 nm. A sample was purified by silica gel chromatography for characterization. **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.40–7.20 (M, 15H), 2.97 (s, 3H), 1.42–1.33 (m, 2H), 1.17–1.07 (m, 2H), 1.00 (s, 3H), 0.81 (t, $J=7.3$ Hz, 3H).

4.6. *tert*-Butyl (2*R*)-2-{(1*R*,2*S*)-2-methoxy-2-methyl-1-[(triphenylmethylsulfenyl)amino]pentyl}-5-oxo-2,5-dihydro-1*H*-pyrrole-1-caboxylate, 4

To a mixture of imine **5** solution (22.25 g by assay, 55.1 mmol), TBSOP[12] **6** solution (23.66 g by assay, 79.6 mmol) and THF (350 mL) at −40 to −45°C was added TfOH (3.74 mL, 42.3 mmol) dropwise such that the internal temp was maintained ≤−35°C. The reaction progress was monitored by HPLC. After 2.5 h, ~5% **5** remained so an additional aliquot of TfOH (0.12 mL, 1.2 mmol) was added. After an additional 1 h the reaction was quenched with 0.5 M $NaHCO_3$ solution (350 mL) and diluted with heptane (84 mL). The mixture was stirred while it warmed to rt, then the layers were separated and the organic portion was washed with 25% wt NaCl solution (84 mL). HPLC assay of the solution showed **4** (31.47 g, 97% yield) and a **4**:**16** isomer ratio of 19.3:1. The solvent was switched to heptane (<1A% THF by GC) by distillation in vacuo while maintaining the volume at ~400 mL until distil-

lation was complete. The product began to crystallize during distillation. The suspension was distilled to a volume of ~240 mL and allowed to cool to rt and stir overnight. The solid was filtered and washed with heptane (2×30 mL). The solid was dried in vacuo at 40°C to give **4** (27.6 g, 85.2% yield, de >99%). Mp 150–152°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, $J=2.0$, 6.1 Hz, 1H), 7.29–7.19 (m, 15H), 6.02 (dd, $J=1.4$, 6.1 Hz, 1H), 4.83 (m, 1H), 3.86 (dd, $J=3.1$, 11.5 Hz, 1H), 3.05 (s, 3H), 2.62 (d, $J=11.2$ Hz, 1H), 1.62–0.98 (m, 4H), 1.36 (s, 9H), 0.92 (t, $J=6.66$ Hz, 3H), 0.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.2, 144.8, 130.1, 127.6, 126.7, 126.6, 82.4, 79.2, 66.9, 6436, 48.9, 38.7, 27.9, 19.3, 17.1, 14.9. HRMS m/z: [M+H] calcd for $C_{35}H_{43}N_2O_4S$, 587.2944; found, 587.2953. Minor diastereomer **16** (oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.18 (m, 16H), 6.02 (dd, $J=1.7$, 6.1 Hz, 1H), 4.90 (m, 1H), 3.89 (dd, $J=2.0$, 11.5 Hz, 1H), 3.06 (s, 3H), 2.42 (d, $J=11.5$ Hz, 1H), 1.54 (s, 9H), 1.28–0.85 (m, 4H), 1.16 (s, 3H), 0.57 (t, $J=7.1$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.2, 129.9, 129.3, 128.1, 127.9, 127.8, 127.7, 127.3, 126.5, 67.2, 65.3, 49.2, 37.3, 28.3, 28.1, 20.9, 17.5, 14.7.

4.7. *tert*-Butyl (2*R*,3*S*)-2-{(1*R*,2*S*)-2-methoxy-2-methyl-1-[(triphenylmethylsulfenyl)amino]pentyl}-5-oxo-3-[(1*Z*)-prop-1-enyl]pyrrolidine-1-caboxylate, 3

A three neck round bottom flask with overhead mechanical stirrer, nitrogen inlet, and thermocouple was evacuated and flushed with nitrogen three times. THF (750 mL) was charged and then the mixture was sparged with nitrogen. $CuBr{\cdot}SMe_2$ (8.74 g, 42.5 mmol) was added and the mixture was chilled to ca. −30°C. A solution of (*Z*)-$CH_3CH{=}CHMgBr$ (0.485 M, 526 mL, 255.3 mmol) was added via cannula maintaining an internal temperature <−20°C (ca. 15 min). TMSCl (11.09 g, 102 mmol) was added and the mixture was stirred 10–15 min while warming to ca. −25°C. A solution of **4** (50 g, 85.1 mmol) in toluene (750 mL) was sparged with nitrogen and then added via cannula to the cuprate reagent solution at such a rate that the internal temperature was ≤−20°C (ca. 20 min). After stirring 5 min, TLC and HPLC analysis showed the consumption of **4**. The reaction was quenched by the addition of NH_4Cl/NH_4OH buffer (600 mL 25% wt NH_4Cl solution+75 mL conc NH_4OH+75 mL water). The nitrogen inlet was removed and the mixture was allowed to warm to rt exposed to air for at least 1 h. The light yellow organic phase was separated from the dark blue aqueous phase. The organics were successively washed with a second portion of the NH_4Cl/NH_4OH buffer (750 mL) and 25% NaCl solution (650 mL). HPLC analyses showed **3** (52.23 g, 83.1 mmol, 97.6% yield) and a *Z*:*E* isomer ratio of 99.4:0.6. This solution was carried on to the next step. A sample was taken for characterization. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.3–7.1 (m, 15H), 5.42–5.36 (m, 2H), 4.00 (s, 1H), 3.69 (t, $J=9.1$ Hz, 1H), 3.59 (dd, $J=2.2$, 10.3 Hz, 1H), 3.06 (d, $J=10.7$ Hz, 1H), 3.03 (s, 3H), 2.81 (dd, $J=9.2$, 17.6 Hz, 1H), 1.95 (dd, $J=1.1$, 17.6 Hz, 1H), 1.58 (d, $J=5.1$ Hz, 3H), 1.49 (m, 1H), 1.46 (m, 1H), 1.44 (s, 9H), 1.12 (m, 1H), 1.05 (m, 1H), 0.86 (t, $J=6.6$ Hz, 3H), 0.52 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 150.9, 144.6, 133.0, 130.0, 127.7, 126.5, 123.1, 82.5, 79.7, 71.7, 66.4, 65.2, 48.8, 39.7, 37.8, 28.0, 27.4, 19.8, 16.9, 14.8, 12.9.

4.8. *tert*-Butyl (2*R*,3*S*)-2-{(1*R*,2*S*)-2-methoxy-2-methyl-1-[(acetylamino)pentyl]-5-oxo-3-[(1*Z*)-prop-1-enyl]-pyrrolidine-1-caboxylate, 20

The solution of crude **3** (50.68 g by assay, 80.6 mmol) was chilled to −20°C and anhydrous HCl (g) (90–100 g) was slowly charged into the solution. The disappearance of **3** was monitored by TLC and HPLC. Upon completion, diatomaceous earth (50 g) was added and the mixture was concentrated in vacuo to ca. one-quarter volume. The mixture was filtered and the vessel and cake were rinsed with water (200 mL) and toluene (200 mL). After separating layers, the organics were extracted with water (100 mL). The combined aqueous portions were washed with toluene (200 mL) and then assayed by HPLC for **18** (20.47 g, 80.5 mmol, 100% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 (br s, 1H), 5.60–5.45 (m, 2H), 3.37 (dd, $J=7.7$, 8.8 Hz, 1H), 3.25–3.15 (m, 1H), 3.16 (s, 3H), 2.80 (d, $J=8.8$ Hz, 1H), 2.42 (dd, $J=8.8$, 16.9 Hz, 1H), 2.10 (dd, $J=10.3$, 16.9 Hz, 1H), 1.70 (d, $J=5.5$ Hz, 3H), 1.65–1.45 (m, 3H), 1.35–1.20 (m, 3H), 1.09 (s, 3H), 0.92 (t, $J=7.3$ Hz, 3H). MS (APCI) m/z 255 $[M+1]^+$. After chilling the aqueous solution to ~10°C, 50% wt NaOH solution (17 mL) was added to adjust the pH to ~12. Ac_2O (16.62 g, 163 mmol) was added; the pH dropped to 6.5 and TLC indicated remaining **18**. Additional 50% wt NaOH solution (4 mL) and Ac_2O (1.48 g, 14.5 mmol) were charged to the reaction mixture; TLC indicated complete consumption of 18. The pH was raised to ~12 with 50% NaOH (2 mL) and NaCl (7 g) was added. The mixture was extracted with *i*-PrOAc (2×200 mL). HPLC analysis of the combined organics for **19** showed 24.85 g (83.8 mmol, 104% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.81 (d, $J=10.6$ Hz, 1H), 5.58–5.35 (m, 2H), 4.25 (dd, $J=8.5$, 10.3 Hz, 1H), 3.52 (dd, $J=7.0$, 8.5 Hz, 1H), 3.29 (m, 1H), 3.18 (s, 3H), 2.44 (dd, $J=9.2$, 16.9 Hz, 1H), 1.98 (dd, $J=9.2$, 16.9 Hz, 1H), 1.95 (s, 3H), 1.62 (dd, $J=1.5$, 6.6 Hz, 3H), 1.50–1.20 (m, 4H), 1.13 (s, 3H), 0.88 (t, $J=6.6$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 169.8, 132.0, 124.9, 79.6, 60.3, 55.4, 48.8, 36.8, 36.7, 36.3, 23.2, 18.8, 15.8, 14.4, 12.9. The solution was azeotropically dried with *i*-PrOAc (3×200 mL) and THF (200 mL) was added. The solution was concentrated and the reconstituted with THF (250 mL). KF analysis showed ≤10 mol% water versus **19**. Et_3N (23.5 mL, 169 mmol), DMAP (0.51 g, 4.2 mmol) and Boc_2O (27.43 g, 125.6 mmol) in THF (20 mL) were added. The reaction was complete after 3.5 h; it was chilled to −10°C and 0.5 M HCl solution (310 mL) was added over 25 min to keep the internal temperature ≤5°C. The mixture was extracted with *i*-PrOAc (2×200 mL). The combined organics were washed with 23% wt NaCl solution (400 mL) and then assayed by HPLC for **20** (28.7 g, 88%

yield). The solution was concentrated in vacuo to ~100 mL, *i*-PrOAc (300 mL) was added to the suspension and the mixture was concentrated again to about one-half volume. Heptane (200 mL) was added to the thick slurry and the mixture was stirred at rt ca. 20 min. The product imide **20** was collected by filtration and dried in vacuo at 50°C to yield 28.24 g (94.9% wt potency, 67.7 mmol, 82% potency adjusted yield from **4**). A sample was recrystallized from MTBE: mp 203–204°C. $[\alpha]_D^{25}$ +48.8 (*c* 1.04 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 5.92 (d, $J=9.6$ Hz, 1H), 5.50–5.40 (m, 2H), 4.54 (dd, $J=2.6$, 9.6 Hz, 1H), 4.01 (d, $J=2.7$ Hz, 1H), 3.80 (t, $J=9.2$ Hz, 1H), 3.23 (s, 3H), 2.76 (dd, $J=9.6$, 18.0 Hz, 1H), 2.06 (dd, $J=1.5$, 18.0 Hz, 1H), 1.98 (s, 3H), 1.77 (m, 1H), 1.64 (dd, $J=1.1$, 6.6 Hz, 3H), 1.63 (m, 1H), 1.56 (s, 9H), 1.31–1.22 (m, 2H), 1.14 (s, 3H), 0.93 (t, $J=7.0$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 170.1, 149.8, 132.5, 123.5, 83.1, 78.7, 64.7, 54.9, 49.0, 39.6, 36.9, 28.1, 27.6, 23.1, 20.5, 17.2, 14.7, 12.9. Anal. calcd for $C_{21}H_{36}N_2O_5$: C, 63.61; H, 9.15; N, 7.06. Found: C, 63.41; H, 9.00; N, 6.95.

4.9. *tert*-Butyl (2*R*,3*S*)-5-methoxy-2-{(1*R*,2*S*)-2-methoxy-2-methyl-1-(acetylamino)pentyl}-3-[(1*Z*)-prop-1-enyl]pyrrolidine-1-carboxylate, 22a

To a solution of **20** (50.0 g, 126 mmol) in THF (500 mL) at ≤−10°C was added a THF solution of $LiEt_3BH$ (1 M, 157 mL, 157 mmol) over ca. 30 min. After stirring briefly, analysis of a sample showed consumption of **20**. MeOH (75 mL) was carefully added and the mixture was then allowed to warm to rt and stir 30 min. The reaction mixture was concentrated to ~200 mL, THF (500 mL) was added, and the solution reconcentrated to ~100 mL. The solution was poured into 5% wt KH_2PO_4 solution (750 mL) and extracted with *i*-PrOAc (750 mL). The layers were separated and the organic portion was washed with ~12% wt NaCl solution (750 mL). HPLC assay showed **21a** 48.42 g (96.6% yield) in solution. ~3:1 mixture of anomers: ^1H NMR (300 MHz, $CDCl_3$) δ 5.92 (d, $J=9.6$ Hz, 1H), 5.75 (dt, $J=1.8$, 10.7 Hz, 1H), 5.45 (dd, $J=1.8$, 6.6 Hz, 0.75H), 5.35–5.41 (m, 1.25H), 4.59 (dd, $J=1.8$, 9.6 Hz, 0.25H), 4.52 (dd, $J=2.6$, 9.6 Hz, 0.75H), 4.18 (m, 1H), 3.90 (m, 0.25H), 3.80–3.70 (m, 1.5H), 3.61 (m, 0.25H), 3.21 (s, 2.25H), 3.20 (s, 0.75H), 2.35–2.20 (m, 1H), 2.00 (s, 2.25H), 1.95 (s, 0.75H), 1.85–1.60 (m, 3H), 1.64 (dd, $J=1.8$, 7.0 Hz, 3H), 1.55 (s, 6.75H), 1.53 (s, 2.25H), 1.35–1.20 (m, 2H), 1.14 (s, 0.75H), 1.12 (s, 2.25H), 0.93 (t, $J=7.3$ Hz, 3H). MS (ESI) m/z 397 $[M-1]^-$. Anal. calcd for $C_{21}H_{38}N_2O_5$: C, 63.29; H, 9.61; N, 7.03; O, 20.07. Found C, 63.45; H, 9.63; N, 7.01; O, 19.88. The solution was concentrated to ~100 mL, diluted with MeOH (500 mL) and reconcentrated (twice). MeOH (90 mL) and $CH_3CH(OCH_3)_3$ (50 mL) were added. The solution was treated with camphorsulfonic acid (1.42 g, 6.1 mmol) and stirred at rt ca. 1 h (no **21a** by TLC). The reaction mixture was poured into 5% wt $KHCO_3$ solution (800 mL) and extracted with *i*-PrOAc (1 L). The organics were washed with 23% wt NaCl solution (500 mL). The organics were concentrated to ~150 mL and diluted with CH_3CN (500 mL). Repeated the concentration/dilution sequence to azeotropically dry the solution (KF <10 mol% water). HPLC assay indicated **22a**, 49.23 g (119.5 mmol, 94.8% yield from 20). ~1:1 mixture of anomers: ^1H NMR (300 MHz, $CDCl_3$) δ 5.98 (d, $J=10.3$ Hz, 1H), 5.49–5.38 (m, 1H), 5.35–5.28 (m, 1H), 5.17 (d, $J=5.5$ Hz, 0.5H), 5.01 (d, $J=5.1$ Hz, 0.5H), 4.72 (dd, $J=2.6$, 9.9 Hz, 1H), 3.93 (dd, $J=2.6$, 7.7 Hz, 0.5H), 3.82 (dd, $J=3.0$, 7.7 Hz, 0.5H), 3.50–3.10 (m, 2H), 3.33 (s, 1.5H), 3.30 (s, 1.5H), 3.06 (s, 3H), 2.05 (s, 1.5H), 2.03 (s, 1.5H), 2.01–1.85 (m, 2H), 1.63 (dd, $J=1.5$, 6.6 Hz, 3H), 1.60–1.40 (m, 3H), 1.56 (s, 4.5H), 1.49 (s, 4.5H), 1.12 (s, 3H), 0.95 (m, 3H). MS (ESI) m/z 413 $[M+1]^+$.

4.10. *tert*-Butyl (2*R*,3*S*,5*R*)-5-cyano-2-{(1*R*,2*S*)-2-methoxy-2-methyl-1-(acetylamino)-pentyl}-3-[(1*Z*)-prop-1-enyl]pyrrolidine-1-carboxylate, 23a

CAUTION: TMSCN is readily hydrolyzed to HCN. Procedures such as described here must be carried out in a well-ventilated fume hood with appropriate safety precautions; see Ref. 29. A crude acetonitrile solution of **22a** (177.5 g solution, 23.83 g by assay, 48.5 mmol) was chilled to −20°C. TMSCN (15.68 g, 158 mmol) was added and the internal temp was lowered to −40°C. TfOH (10.91 g, 72.7 mmol) was added over 30 min. The reaction was stirred at −40°C for 2.5 h at which point HPLC analysis of a sample indicated that the reaction was complete. The reaction was quenched into a mixture of 10% wt K_2CO_3 (400 mL) with NaOH (7.8 g) and *i*-PrOAc (400 mL); pH of aqueous ~11.2. The organic portion was washed with 23% wt NaCl solution (200 mL), pH of aq ~9.6; then with 23% wt NaCl solution (200 mL) containing KH_2PO_4 (2.8 g) and $Na_2HPO_4{\cdot}7H_2O$ (7.1 g), pH of aq ~6.2. After solvent switching to *i*-PrOH, HPLC assay showed the desired α-isomer **23a** (16.49 g, 40.5 mmol, 83.5% yield) and undesired β-isomer **24a** (0.67 g, 1.6 mmol, 3.4% yield); α:β ratio 24.6:1. **23a**: ^1H NMR (500 MHz, C_6D_6) δ 5.77 (m, 1H), 5.38 (d, $J=9.3$ Hz, 1H), 5.28 (dq, $J=13.7$, 7.1 Hz, 1H), 4.86 (dd, $J=1.3$, 9.4 Hz, 1H), 4.07 (d, $J=9.3$ Hz, 1H), 4.04 (s, 1H), 3.56 (dd, $J=8.1$, 10.4 Hz, 1H), 2.79 (s, 3H), 2.12 (m, 1H), 1.98 (ddd, $J=8.2$, 9.3, 12.4 Hz, 1H), 1.63 (d, $J=13.4$ Hz, 1H), 1.60 (s, 9H), 1.55–1.48 (m, 4H), 1.46 (s, 3H), 1.26 (m, 1H), 1.07 (m, 1H), 0.90 (t, $J=7.1$ Hz, 3H), 0.85 (s, 3H). ^{13}C NMR (125 MHz, C_6D_6) δ 168.5, 152.8, 132.7, 123.3, 120.5, 81.2, 79.3, 65.2, 54.2, 48.5, 46.8, 37.9, 37.4, 36.1, 28.4, 22.8, 20.2, 17.1, 15.1, 12.8. **24a**: ^1H NMR (500 MHz, C_6D_6) δ 5.85 (d, $J=9.8$ Hz, 1H), 5.26 (m, 1H), 5.06 (dd, $J=2.3$, 9.8 Hz, 1H), 4.92 (m, 1H), 4.09 (dd, $J=4.3$, 8.5 Hz, 1H), 3.83 (dd, $J=2.5$, 5.0 Hz, 1H), 3.53 (m, 1H), 2.80 (s, 3H), 2.03 (m, 1H), 1.92 (s, 3H), 1.88 (m, 1H), 1.55 (s, 9H), 1.48 (dd, $J=1.7$, 6.8 Hz, 3H), 1.42 (m, 1H), 1.30–1.18 (m, 3H), 0.96 (s, 3H), 0.91 (t, $J=7.0$ Hz, 3H). ^{13}C NMR (125 MHz, C_6D_6) δ 169.8, 153.0, 132.3, 124.1, 119.3, 81.2, 79.2, 65.0, 53.3, 48.1, 47.2, 37.7, 37.2, 36.1, 28.2, 23.4, 20.2, 16.9, 14.8, 12.7. Elimination side product, **25**. ~1:1 *N*-Boc rotomers: ^1H NMR (300 MHz, $CDCl_3$) δ 6.61 (dd, $J=1.1$ Hz, 4.0, 0.5H), 6.43 (dd, $J=1.5$ Hz, 4.4, 0.5H), 5.88 (d, $J=9.9$ Hz, 1H), 5.47–5.33 (m, 1H), 5.26 (t, $J=10.6$, 1H), 4.73 (dd,

$J=2.9$ Hz, 4.4, 0.5H), 4.68 (d, $J=2.2$ Hz, 0.5H), 4.64 (dd, $J=2.9$ Hz, 4.0, 0.5H), 4.60 (d, $J=2.6$ Hz, 0.5H), 4.29 (d, $J=9.6$ Hz, 0.5H) 4.13 (d, $J=9.9$ Hz, 0.5H), 4.09 (t, $J=2.6$, 0.5H), 3.95 (t, $J=2.6$ Hz, 0.5H), 3.19 (s, 1.5H), 3.15 (s, 1.5H), 1.96 (s, 1.5H), 1.95 (s, 1.5H), 1.77–1.60 (m, 2H), 1.68 (dd, $J=1.5$ Hz, 6.6, 3H), 1.55 (s, 4.5H), 1.49 (s, 4.5H), 1.44–1.20 (m, 2H), 1.14 (s, 1.5H), 1.15 (s, 1.5H), 0.95 (dt, $J=1.1$ Hz, 6.6, 3H). MS (ESI) m/z 381 $[M+1]^+$.

4.11. Isopropyl (2*R*,4*S*,5*R*)-5-[(1*R*,2*S*)-1-(acetylamino)-2-methoxy-2-methylpentyl}-4-[(1*Z*)-prop-1-enyl]-pyrrolidine-2-carboxylate, A-322278·TsOH 2·TsOH

An *i*-PrOH solution of crude 2-cyanopyrrolidines (231 g solution containing **23a** (46.7 g, 114.7 mmol) and **24a** (2.1 g, 5.2 mmol)) was chilled to ≤ -30°C with a dry ice/MeOH bath and carefully treated with anhydrous HCl (g) at such a rate to maintain an internal temp of <0°C until the exotherm ceased (ca. 100 g HCl added). After the addition was complete, the reaction was allowed to warm while stirring to $\sim +15$°C. HPLC analysis showed that starting materials **23a**/**24a** and intermediate **29** had been consumed. The reaction mixture was diluted with *i*-PrOH (235 g) and warmed to reflux for 8 h. After cooling to rt, the reaction was concentrated in vacuo to about one-half volume. It was replenished with *i*-PrOAc and reconcentrated. The mixture was poured into 15% wt $KHCO_3$ solution (690 g) and the reaction flask rinses, *i*-PrOAc (250 g) and water (50 g), were also added. After through mixing, the layers were separated. The organic portion was washed with 15% wt $KHCO_3$ solution (380 g) and the combined aqueous portions were back-extracted with *i*-PrOAc (160 g). The combined organics were washed with 20% wt NaCl solution (300 g); KF of the organic portion was $\sim 2.4\%$ wt water. The organics were azeotropically dried with *i*-PrOAc (to KF <0.05% wt water). The mixture was filtered to removed precipitated inorganic salts and the filtrate was assayed by HPLC for **2** (37.2 g, 101 mmol, 88% yield) and the α/β ratio was 12.0:1. The filtrate solution was diluted with *i*-PrOAc to give 1500 g solution and then it was warmed to 50–60°C. $TsOH\cdot H_2O$ (24.07 g, 126.6 mmol) was dissolved in *i*-PrOAc (256 g) by warming to 50–60°C; this solution was added to the solution of **2** through an in-line filter. Within minutes, a white precipitate began to form; after ca. 1 h at 50–60°C, the mixture was cooled to rt at 5°C/h and allowed to stir at rt several h. The product was collected by filtration and dried in vacuo to provide **2**·TsOH (53.78 g, 96.7% potency, 96.2 mmol, 84% yield from **23a**). mp 207–208°C. $[\alpha]_D^{25}$ −31.4 (*c* 1.04, MeOH). ^{1}H NMR (300 MHz, D_6-DMSO) δ 9.08 (br s, 1H), 8.74 (br s, 1H), 7.66 (d, $J=10.3$ Hz, 1H), 7.48 (d, $J=8.1$ Hz, 2H), 7.12 (d, $J=8.1$ Hz, 2H), 5.45 (dq, $J=11.0$, 6.6 Hz, 1H), 5.28 (ddq, $J=8.8$, 11.0, 1.5 Hz, 1H), 5.01 (spt, $J=6.3$ Hz, 1H), 4.40–4.28 (m, 2H), 3.60 (t, $J=9.6$ Hz, 1H), 3.36 (br s, 1H), 3.20–3.10 (m, 1H), 3.13 (s, 3H), 2.43 (dt, $J=12.9$, 8.1 Hz, 1H), 2.29 (s, 3H), 1.79 (s, 3H), 1.60–1.40 (m, 2H), 1.52 (dd, $J=1.5$, 6.6 Hz, 3H), 1.30–1.10 (m, 2H), 1.25 (d, $J=6.3$ Hz, 3H), 1.23 (d, $J=6.3$ Hz, 3H), 1.18 (s, 3H), 0.80 (t, $J=7.0$ Hz, 3H). ^{13}C NMR (75 MHz, D_6-DMSO) δ 169.6, 168.3, 145.7, 137.6, 130.3, 128.0, 125.5, 124.8, 79.4, 70.0, 63.0, 58.9, 52.3, 48.7, 35.9, 33.8, 22.4, 21.4, 21.3, 20.8, 18.0, 15.1, 14.3, 12.7. Anal. calcd for $C_{27}H_{44}N_2O_7S$: C, 59.97; H, 8.20; N, 5.18; O, 20.71; S, 5.93. Found: C, 59.91; H, 8.08; N, 5.15; S, 5.94. **Diketopiperazine, 26**. ^{1}H NMR (300 MHz, CD_3OD) δ 5.38 (s, 2H), 5.29 (dq, $J=6.6$, 10.6 Hz, 2H), 5.20 (ddq, $J=8.8$, 10.6, 1.5 Hz, 2H), 3.98 (d, $J=8.8$ Hz, 2H), 3.55 (t, $J=7.7$ Hz, 2H), 3.10 (s, 6H), 3.02 (dd, $J=7.0$, 8.5 Hz, 2H), 2.83 (m, 2H), 2.19 (dt, $J=12.5$, 7.7 Hz, 2H), 1.80 (s, 6H), 1.48 (dd, $J=1.5$, 6.6 Hz, 6H), 1.45–1.20 (m, 6H), 1.12 (s, 6H), 1.10–0.95 (m, 4H), 0.77 (t, $J=7.0$ Hz, 6H). MS (ESI) m/z 617.6 $[M+1]^+$.

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29. **CAUTION**: It is worth noting the safety precautions taken when conducting this reaction. The reaction and work up were executed with a nitrogen sweep through the headspace; the outlet was connected to a caustic (NaOH) scrubber solution with an intervening trap to protect against the scrub solution backing-up into the vessel. After the reaction, the mixture was quenched into a 10% wt potassium carbonate solution containing sodium hydroxide (ca. 1.4 equiv. versus TMSCN used) to maintain a pH >11 (the pK_a of HCN is 9.2, the pH of a 0.1N solution of HCN is 5.1). The aqueous waste steam and scrubber solutions were treated with bleach (sodium hypochlorite) to oxidize excess cyanide (see Lunn, G.; Sanaone, E. B. *Destruction of Hazardous Chemicals in the Laboratory*; John Wiley & Sons: New York, 1994, 133–138.) The cyanide levels were tested with ion specific test strips (EM QUANT by E-Merck, item number EM-10044-1 from VWR Scientific Products) prior to disposal to insure complete cyanide ion oxidation.
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