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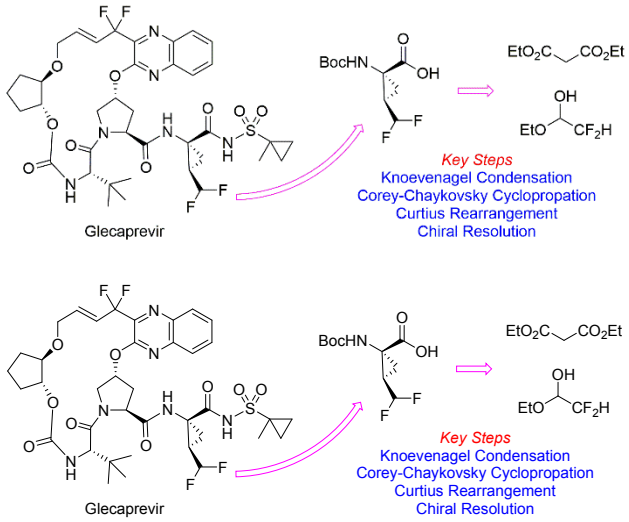
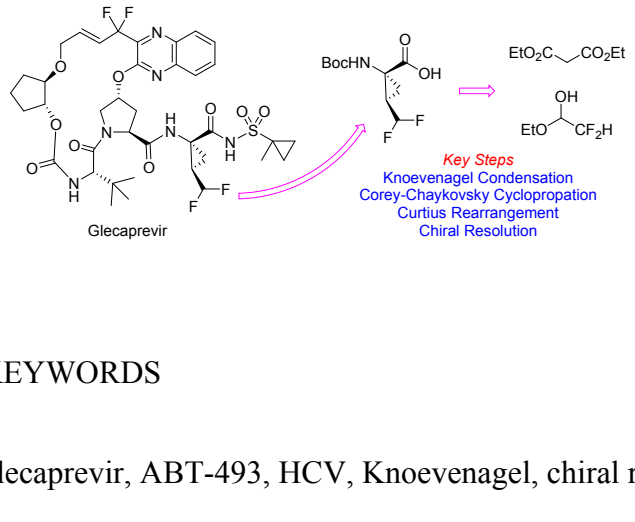




KEYWORDS

glecaprevir, ABT-493, HCV, Knoevenagel, chiral resolution

Abstract: The preceding manuscript described the development of the large-scale synthetic route to macrocycle **3** of glecaprevir (**1**), a potent HCV protease inhibitor. This manuscript describes the development of the synthesis of the difluoromethyl substituted cyclopropyl amino acid **4**, its conversion to the fully elaborated sidechain, amino sulfonamide **2**, and the subsequent final coupling to form glecaprevir. The synthesis of amino acid **4** consists of four key transformations (a) formation of the difluoromethyl substituted cyclopropane ring of (±)-diester **15** via Knoevenagel condensation and Corey-Chaykovsky cyclopropanation, (b) diastereoselective hydrolysis of (±)-diester **15** to yield the (±)-monoacid **14a-b**, (c) conversion of (±)-monoacid **14a-b** to (±)-amino ester **10** via a Curtius rearrangement, and (d) resolution of (±)-amino ester **10** followed by saponification to the desired (1*R*,2*R*)-amino acid **4**. The large-scale synthetic route to amino acid **4** was successfully used to produce the fully elaborated sidechain, amino sulfonamide **2**, and ultimately the amount of glecaprevir required to support the late stage clinical development.

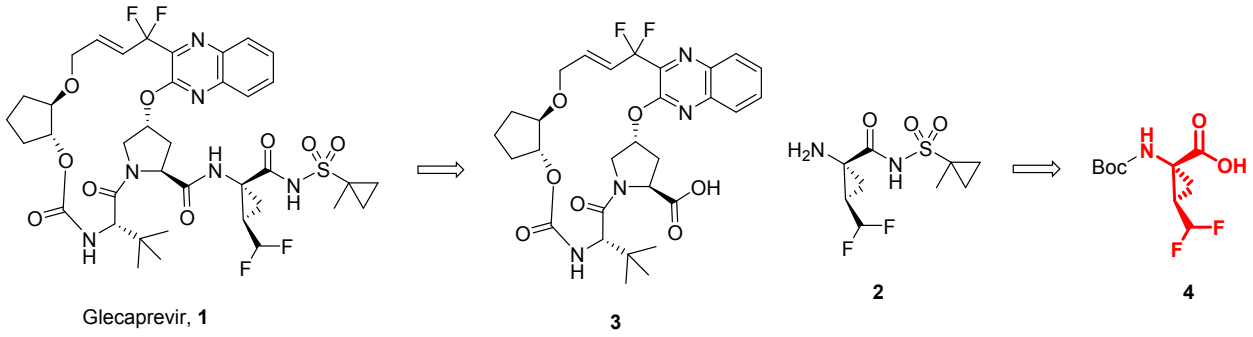
Introduction

Glecaprevir was identified as a potent HCV NS3/4A protease inhibitor, and an enabling synthesis was used to support the early clinical development.[1] The enabling synthesis was deemed unsuitable for the large-scale production required for late stage clinical development. As described in the preceding manuscript, a new synthetic route to glecaprevir was required to support the transition to late stage clinical development.[2]

The initial disconnection in the retrosynthetic analysis for both the enabling route and the large-scale route for glecaprevir (**1**), shown in Scheme 1, dissects glecaprevir into macrocycle **3** and amino sulfonamide **2**, and ultimately to amino acid **4**. The preceding manuscript addressed the first limitation of the enabling route to glecaprevir; the large-scale synthesis of macrocycle

3.[2] This manuscript is focused on the second limitation of the enabling route; the efficiency of the synthesis of the difluoromethyl substituted cyclopropyl amino acid **4**, and in particular overcoming the challenging fluorination step that was the key step in the enabling synthesis.[3]

Scheme 1: Retrosynthetic Analysis to Glecaprevir



The synthetically challenging structural features to be addressed in the development of a new synthesis of amino acid **4** are highlighted in red in Scheme 1. Although amino acid **4** contributes only five carbon atoms to the core of glecaprevir, it contains considerable structural complexity: two stereocenters, a cyclopropane ring, an amino acid, and a difluoromethyl group. A new synthesis of amino acid **4** would require concise installation of these structural features while ensuring stereochemical control. With these objectives in mind, the development of a large-scale synthetic route to amino acid **4** began.

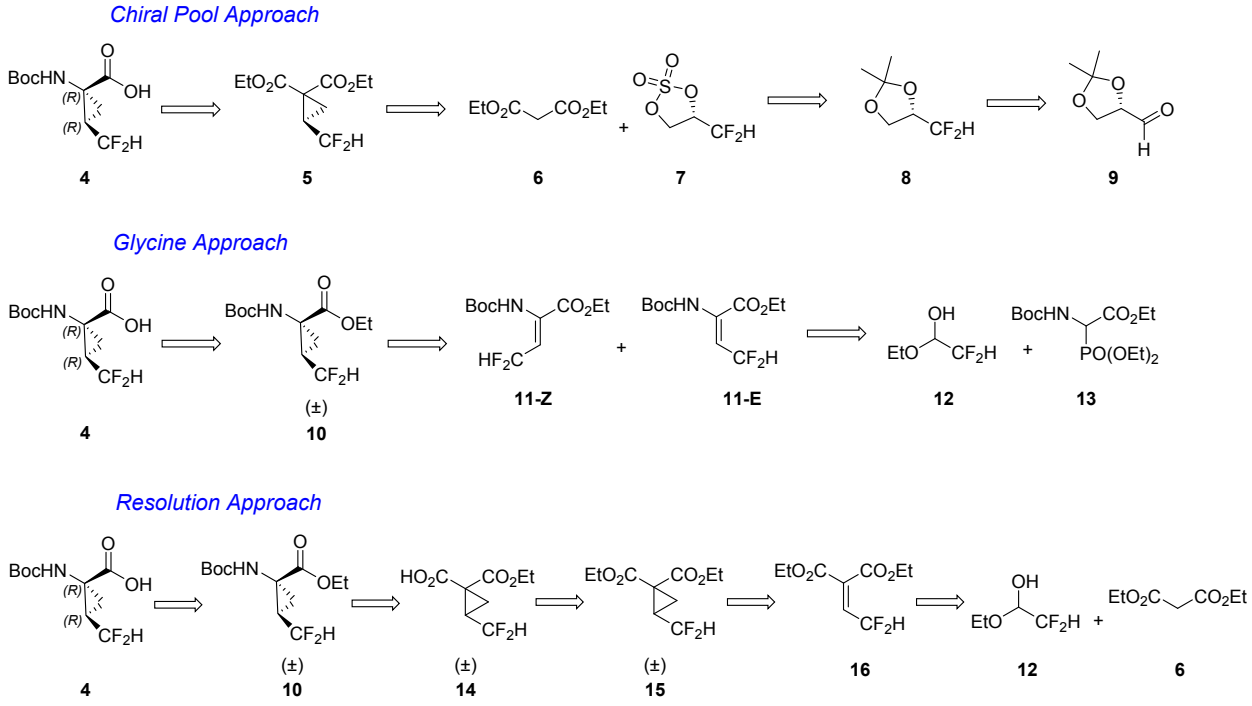
Results and Discussion

Retrosynthetic Analysis of Amino Acid 4

Numerous routes to amino acid **4** were proposed and multiple approaches were evaluated experimentally. The potential routes are outlined in Scheme 2, and are exemplary of the challenges to be addressed in the development of a new route to amino acid **4**. The chiral pool approach commences with the fluorination of (*S*)-glyceraldehyde acetonide **9** to form the known difluoromethyl acetonide **8**.[4] While this fluorination of **9** proceeds in excellent yield, the use of

diethylaminosulfur trifluoride (DAST) or a similar reagent presents challenges in terms of safety and availability for large-scale production.[5] The conversion of **8** to the cyclic sulfate **7**, followed by reaction with diethyl malonate **6** to form cyclopropane **5** was expected to proceed smoothly.[6] In practice, cyclopropane **5** was successfully synthesized, however cyclic sulfate **7** exhibited limited stability. In addition to the safety concerns with the fluorination step and stability concerns with cyclic sulfate **7**, the chiral pool approach would result in a ten-step synthesis which would not be an improvement over the enabling route. The chiral pool approach and other routes that involved fluorination were deprioritized due to the safety concerns, cost, and limited availability on scale of many fluorination reagents.

Scheme 2: Potential Routes to Amino Acid **4**



Routes starting from fluorinated materials that were commercially available on large-scale were prioritized. Using this strategy, difluoroacetaldehyde ethyl hemiacetal **12** (Scheme 2) emerged as a key building block for amino acid **4**. The hemiacetal **12** is available by reduction of

ethyl difluoroacetate and is readily transformed into a variety of substrates.[7] This strategy aligns with the glycine approach, shown in Scheme 2, which begins with hemiacetal **12** and allows the potential to construct the cyclopropane ring of **10** with the requisite amino acid functionality. The challenge with the glycine approach lies in the control of the alkene geometry of compound **11**. Based on literature precedent from Knowles, the Horner-Wadsworth-Emmons (HWE) olefination to form alkene **11** was expected to adopt the Z configuration, leading to the incorrect stereochemistry upon cyclopropanation.[8] Indeed, the syntheses of structurally related difluoromethyl alkenes were found to favor the Z configuration, leading to the incorrect stereochemistry upon cyclopropanation.[9] A brief evaluation of chiral auxiliaries to favor the E alkene configuration in the synthesis of analogs of **11** proved low yielding at best, confirming the expected selectivity challenges with the glycine approach.[10]

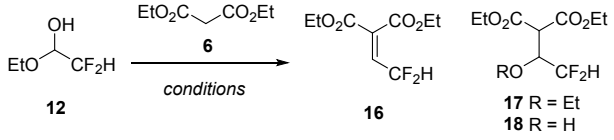
With the considerations for stereochemical control and the source of the difluoromethyl functionality in mind, the resolution approach for the synthesis of amino acid **4** was developed, as shown in Scheme 2. Starting from hemiacetal **12**, a Knoevenagel condensation of diethyl malonate **6** would yield alkylidene **16**. Cyclopropanation of alkylidene **16** to form the racemic diester **15**, followed by selective saponification of the least sterically hindered ester would set the relative stereochemistry of the cyclopropane ring of racemic monoacid **14**. A Curtius rearrangement of the racemic monoacid **14** would produce the racemic ester **10**. The desired (1*R*,2*R*) configuration of amino acid **4** could be derived through resolution of the corresponding racemic ester **10**. While the resolution approach requires a selective diester hydrolysis and a Curtius reaction not required in the glycine approach, these transformations were expected to proceed in acceptable yield and selectivity. Alternatively, reversing the selectivity of the HWE

reaction to favor **11-E** (or analog) in the glycine approach was deemed to have a low probability success. For these reasons, the resolution approach was selected for further development.

Synthesis of (±)-Diester 15

The Knoevenagel condensation of diethyl malonate **6** with difluoroacetaldehyde ethyl hemiacetal **12** proved to be a challenging reaction as the product distribution was found to vary significantly depending on the choice of Lewis acid and solvent.[11] As shown in Table 1, the reaction produced the desired alkylidene **16**, along with varying amounts of ethanolate **17** and hydrate **18** due to the exceptionally electrophilic nature of alkylidene **16**. The ratio of **16** and **17** was inconsequential as both were competent substrates in the subsequent cyclopropanation reaction.

Table 1: Screen of Knoevenagel Reaction Conditions



		GC area %			
Lewis Acid	Conditions	**6**	**16**	**17**	**18**
$TiCl_4$	a	1.7	75.0	8.2	2.3
$TiCl(OEt)_3$	b	0.1	68.3	22.8	2.3
$CeCl_3$, NaI	c	5.5	3.6	90.5	0.6
$Ce_2(SO_4)_3$, NaI	d	4.6	4.0	89.6	1.2
LiCl	e	88.2	3.0	6.1	2.4
$MgCl_2$	f	4.0	4.1	90.6	1.1

(a) 2.0 equiv each of $TiCl_4$ and Et_3N, 1.0 equiv each of **6** and **12**, THF (25 mL/g of **6**), 0 °C to rt, 22 h. (b) 2.0 equiv each of $TiCl(OEt)_3$ and Et_3N, 1.0 equiv each of **6** and **12**, THF (21.5 mL/g of **6**), 0 °C to rt, 63 h. (c) 0.02 equiv each of $CeCl_3$ and NaI, 1.0 equiv **6**, 1.2 equiv **12**, EtOH (4 mL/g of **6**), 60 °C, 5 h. (d) 0.05 equiv each of $Ce_2(SO_4)_3$ and NaI, 1.0 equiv **6**, 1.2 equiv **12**, EtOH (4 mL/g of **6**), 60 °C, 72 h. (e) 0.05 equiv LiCl, 1.0 equiv **6**, 1.2 equiv **12**, EtOH (4 mL/g of **6**), 60 °C, 22 h. (f) 0.05 equiv $MgCl_2$, 1.0 equiv **6**, 1.2 equiv **12**, EtOH (4 mL/g of **6**), 60 °C, 22 h.

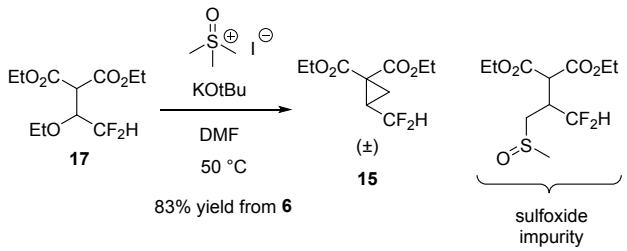
The use of $TiCl_4$ with Et_3N in THF resulted in high conversion to **16** and **17**, but also formed several minor impurities. Further development revealed the combination of $TiCl(OEt)_3$ and Et_3N

in THF produced a 3:1 mixture of alkylidine **16** and ethanolate **17** in a combined 90% by GC analysis. Although $TiCl(OEt)_3$ worked well in the condensation reaction, a stoichiometric quantity of this expensive reagent would be needed for large scale manufacture. In addition, the reaction work-up was cumbersome and generated a significant amount of titanium waste. Several alternative Lewis acids were screened, as shown in Table 1. The combination of $CeCl_3$ and NaI (2 mol % each) resulted in essentially complete conversion after 5 h at 60 °C in EtOH.[12] The switch to EtOH as the solvent resulted in formation of ethanolate **17** as the main product. Cerium sulfate with NaI resulted in a much slower conversion, and LiCl resulted in minimal conversion. A catalytic amount of magnesium chloride (5 mol %) in ethanol was slightly slower than $CeCl_3$ but was found to be desirable from a reaction profile, scale-up and commercialization perspective. Accordingly, diethyl malonate **6** was treated with hemiacetal **12** and magnesium chloride in ethanol yielding a mixture containing ethoxy adduct **17** as the main product. A solvent switch to MTBE allowed removal of the magnesium chloride by filtration, yielding a product solution suitable for use in the subsequent cyclopropanation.

A brief screen of reaction conditions revealed that the Corey-Chaykovsky cyclopropanation was optimal for the synthesis of (±)-diester **15**, as shown in Scheme 3.[13] A detailed safety evaluation for this reaction has been reported, and DMF was identified as the preferred solvent for this reaction instead of the more commonly used DMSO.[14] A minor side product was identified as the sulfoxide impurity, formed by demethylation of the intermediate rather than cyclization to (±)-diester **15**. The slow, simultaneous addition of separate solutions of substrate **17** and the ylide to a heated DMF solution was found to minimize the formation of the sulfoxide impurity. This approach results in a steady low concentration of the intermediate favoring cyclization over demethylation. The process allowed the isolation of (±)-diester **15** in 93% purity

after an aqueous workup. The two-step process of Knoevenagel condensation and Corey-Chaykovsky cyclopropanation was successfully scaled to >400 kg for the production of (±)-diester **15** in 83% overall yield from **6**.

Scheme 3: Corey-Chaykovsky Cyclopropanation of **17**

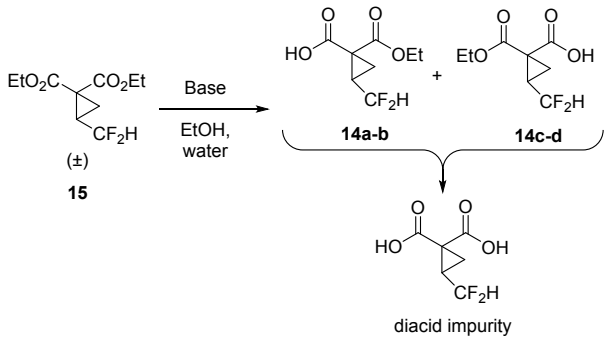


Synthesis of (±)-Monoacid 14a-b and Determination of Stereochemistry

The next step in the synthesis of amino acid **4** was to selectively hydrolyze the least sterically hindered ester of (±)-diester **15** to produce the desired diastereomer of (±)-monoacid **14**. A screen of hydrolysis conditions was conducted (Table 2) to identify conditions favoring the desired diastereomer **14a-b** over **14c-d**. The reaction was also found to generate varying levels of the diacid impurity. Lithium hydroxide and potassium hydroxide generated unacceptably high levels of the diacid impurity, even with incomplete conversion of the starting material. The use of quaternary ammonium hydroxides resulted in a dramatic reduction in the amount of diacid impurity while achieving essentially full conversion of the starting material. Tetra-*n*-butylammonium hydroxide was selected as the preferred base due to the high conversion and low diacid impurity levels. The amount of diacid impurity was further controlled by limiting the hydroxide charge to the minimum required to complete the saponification. The ethanol:water ratio was found to have a modest effect on the diastereoselectivity, with 30-45% ethanol producing the highest level of **14a-b**. At higher water content the reactions produced higher levels of diacid with slightly more residual starting material. A solvent screen (THF, MeCN, 1,4-

dioxane, DMSO, DME, *t*-BuOH) was conducted but no improvement in selectivity was observed over ethanol. Minimal variation in the product distribution was observed from -10 to 45 °C. The reaction temperature was set at 0 to 5 °C in order to minimize diacid formation upon extended reaction times.

Table 2: Screen of Diester Hydrolysis Conditions



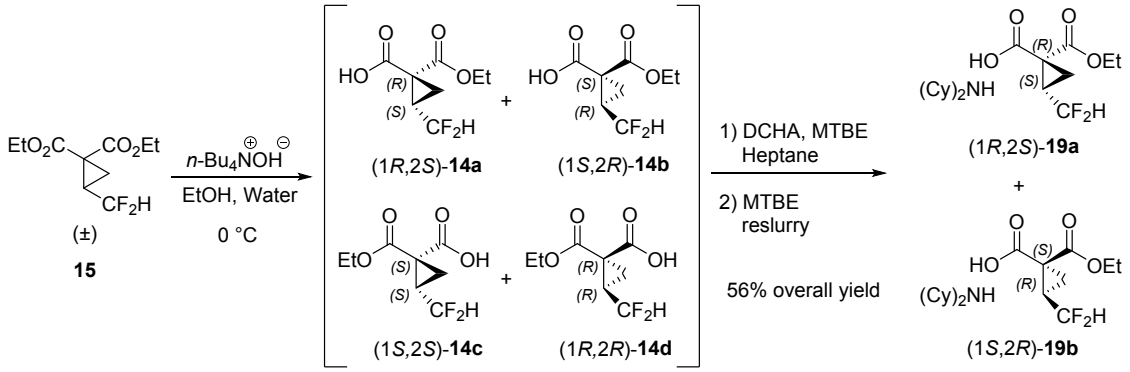
Base (Equiv)	EtOH [a] (vol %)	HPLC area % 15	14a-b	14c-d	diacid
LiOH (1.2)	83	11.5	68.3 [b]		17.4
KOH (1.0)	83	7.7	82.1 [b]		7.7
Me_4NOH (1.0)	78	1.4	73.7	19.7 [c]	2.8
$BnMe_3NOH$ (1.1)	81	1.5	75.5	20.7 [c]	2.3
n-Bu_4NOH (1.04)	84	0	74.9	23.6	1.5
n-Bu_4NOH (1.09)	50	0	77.5	18.0	4.5
n-Bu_4NOH (1.05)	42	0	78.7	18.7	2.5
n-Bu_4NOH (1.07)	34	0.5	78.8	18.8	1.9
n-Bu_4NOH (1.09)	17	1.3	78.4	16.7	3.6
n-Bu_4NOH (1.20)	11	4.2	75.9	16.1 [c]	3.8

(a) Total solvent volume at 5.9 to 6.9 mL/g of **15**. Reactions cooled below 15 °C prior to base addition and then warmed to rt. (b) Total of **14a-b** and **14c-d**. (c) Ratio of **14a-b** to **14c-d** determined by H^1 NMR.

As shown in Scheme 4, the hydrolysis of **15** theoretically results in four isomers **14a-d**, two diastereomeric pairs of enantiomers. The best hydrolysis conditions identified in Table 2 resulted in a 4 to 1 ratio of **14a-b** *vs* **14c-d**. Thus, attention shifted to removal of undesired isomers **14c-d**. It was found that treatment of the mixture of **14a-d** with dicyclohexylamine (DCHA, $(Cy)_2NH$)

in MTBE and heptane not only produced crystalline DCHA salt **19a-b** but improved the diastereomeric ratio to >9:1 of **14a-b** *vs* **14c-d**. Encouraged by these results, it was found that the minor diastereomer **14c-d** could be reduced to less than 1% by a subsequent reslurry of the DHCA salt in MTBE. This low level of **14c-d** was readily rejected in the downstream steps. The DCHA salt crystallization also resulted in complete rejection of the diacid impurity. The hydrolysis and crystallization process outlined in Scheme 4 produced the DCHA salt **19a-b** in a moderate 56% yield from **15**. The diastereoselectivity of the hydrolysis reaction (4:1) resulted in the moderate yield for this step and was identified as an area for future process development.

Scheme 4: Synthesis of (±)-Monoacid DCHA Salt **19a-b**



Although the major diastereomer **14a-b** was successfully isolated in >99% purity as its DCHA salt **19a-b**, the desired absolute stereochemistry still needed to be established. In this context, the four isomers **14a-d** were separated by preparative HPLC (see Figure 1) in high purity. The individual isomers **14a-d** were converted to their corresponding DCHA salts **19a-d** and analyzed by chiral HPLC for purity, which is summarized Table 3. The absolute stereochemistry of three of the DCHA salts **19a-d** was determined by single crystal X-ray analysis and it was found (Table 3, Figure 2) that the peak at 11.65 min was the desired (1*S*,2*R*)-**14b** isomer.

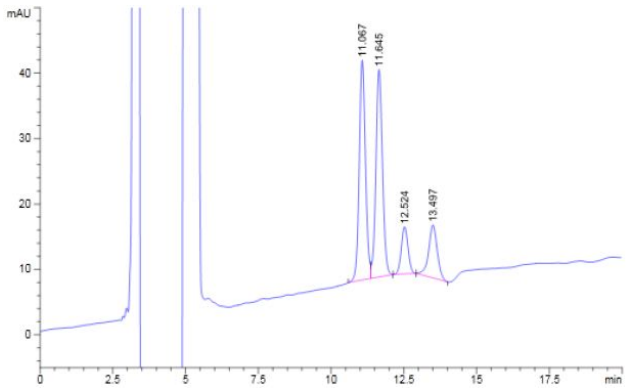


Figure 1: Chiral HPLC of Mixture of Monoacid Isomers **14a-d**

Table 3: Chiral HPLC of Isolated Monoacid Isomers **14a-d** and Absolute Stereochemistry

Entry	Monoacid **14a-d**	ee	de	Configuration
1	**14a**, Peak at 11.07 min	>99.9%	>99.9%	(1*R*,2*S*)
2	**14b**, Peak at 11.65 min	>99.9%	98.6%	(1*S*,2*R*)
3	**14c**, Peak at 12.52 min	>99.9%	92.6%	(1*S*,2*S*)
4	**14d**, Peak at 13.50 min	>99.9%	>99.9%	(1*R*,2*R*)

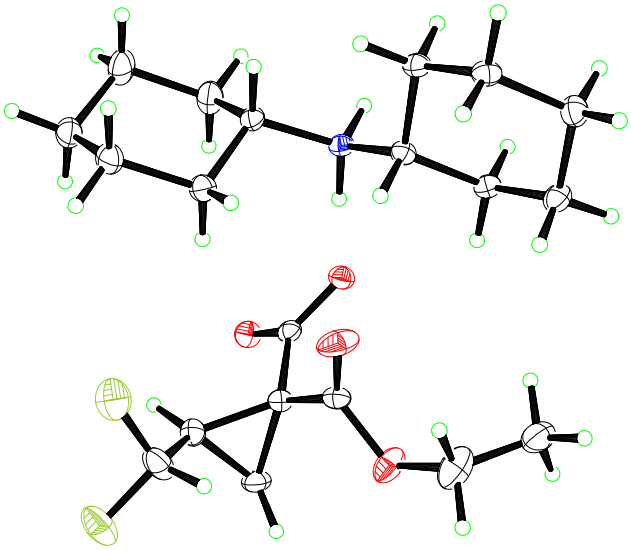


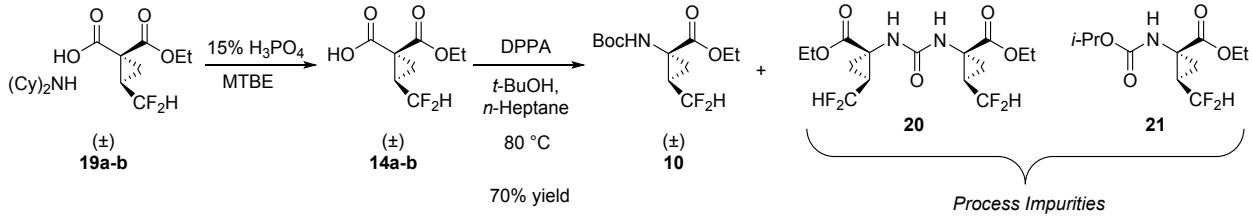
Figure 2: Structure from Single Crystal X-Ray of (1*S*,2*R*)-Monoacid DCHA Salt **19b**

Synthesis of (±)-Amino Ester 10

The next step in the synthesis of amino acid **4** was the Curtius rearrangement of (±) monoacid **14a-b** to the (±)-amino ester **10**, as shown in Scheme 5.[15] A thorough safety assessment of the Curtius reaction revealed the adiabatic temperature rise was approximately 40 °C, and the

reaction generated approximately 100 L of nitrogen per kg of **14a-b**. In order to prevent a buildup of the intermediate acyl azide and control gas generation, the process design incorporated the slow addition of diphenylphosphoryl azide (DPPA) to the acid **14a-b** at approximately 80 °C. Using this approach, as it forms the acyl azide immediately undergoes the Curtius rearrangement and subsequent trapping by *tert*-butanol to produce Boc protected amino ester **10**. The reaction generated several impurities including the two shown in Scheme 5 (**20** and **21**). The presence of water in the reaction resulted in hydrolysis of the intermediate isocyanate and led to the formation of dimeric urea impurity **20** (only one stereoisomer is shown but a pair of diastereomeric urea impurities was formed). The presence of other alcohols, either as impurities in *tert*-butanol or in the reactor train from processing or cleaning, resulted in the formation of carbamate impurities such as **21** derived from isopropanol. Rigorous exclusion of moisture and other alcohols from the reactor train, along with the use of high purity *tert*-butanol, minimized the formation of impurities such as urea **20** and carbamate **21**. As shown in Scheme 5, after a salt break, the free acid **14a-b** was converted to (±)-amino ester **10** through the Curtius reaction. After an aqueous workup, the product was crystallized from heptane in 70% yield. This transformation was successfully scaled to >200 kg in a reproducible yield and quality of (±)-amino ester **10** over multiple batches.

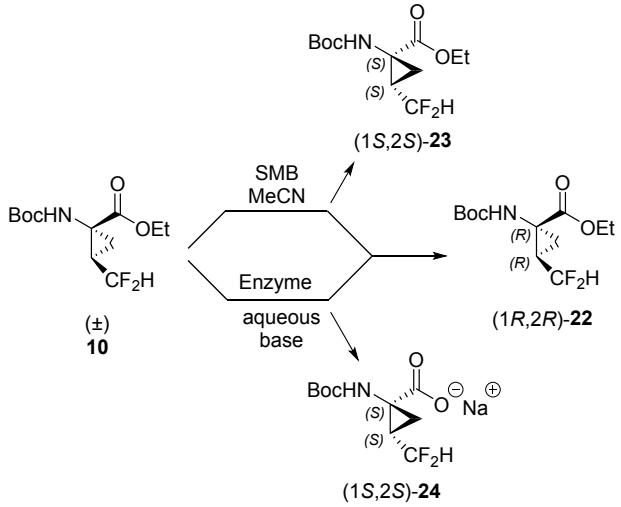
Scheme 5: Synthesis of (±)-Amino Ester **10**



Resolution of (±)-Amino Ester 10

Two complimentary methods were developed for the resolution of (±)-amino ester **10**, as outlined in Scheme 6. One of the unique aspects is that the two different resolution methods allow for isolation of the same product, (1*R*,2*R*)-amino ester **22**. One method developed for resolution of the (1*R*,2*R*) and (1*S*,2*S*) enantiomers (**22** and **23**) was simulated moving bed (SMB) chromatography.[16] The SMB chromatography utilized Chiralpak AY (particle size: 20 μm) as the stationary phase and involved preparation of a feed stock solution of (±)-amino ester **10** in acetonitrile at a concentration of approximately 50 g/L. The chiral purity of the raffinate (product fraction) was analyzed at 99% ee for (1*R*,2*R*)-amino ester **22** and was isolated in 41-47% yield (out of a theoretical 50%) as an acetonitrile solution.

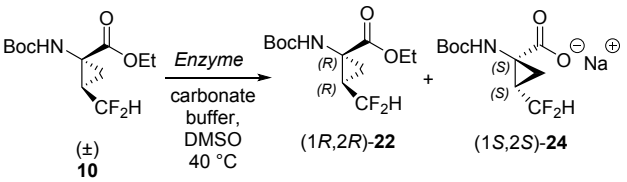
Scheme 6: Resolution Methods for (±)-Amino Ester **10**



The other approach developed was an enzymatic resolution of (±)-amino ester **10**, as shown in Scheme 6.[17] An enzyme screening study for the resolution of (±)-amino ester **10** was carried out evaluating sixteen commercially available enzymes. Experiments with lipases and esterases showed no conversion. The three protease enzymes in Table 4 showed conversion with hydrolysis to the undesired (1*S*,2*S*)-amino acid **24**, yielding the (1*R*,2*R*)-amino ester **22** in greater

than 96% enantiomeric excess. These results demonstrated that the resolution could selectively hydrolyze the undesired enantiomer allowing separation of the desired (1*R*,2*R*)-amino ester **22** isomer from the (1*S*,2*S*)-amino acid **24** carboxylate salt through an aqueous workup. From this initial screening study (Table 4), it was found that the reaction rates with Alcalase, Esterase, and Savinase were comparable, but Alcalase 2.4L (Entry 1) produced (1*R*,2*R*)-amino ester **22** with the highest chiral purity. Therefore, Alcalase 2.4L was selected for further development.

Table 4: Enzyme Screen for Resolution of (±)-Amino Ester **10**



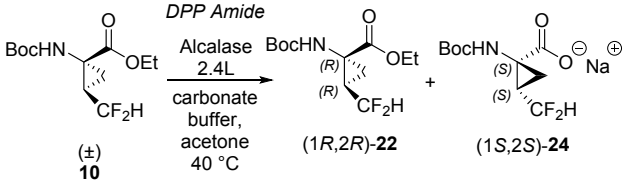
Entry	Enzyme	(1*R*,2*R*)-Amino Ester **22** ee
1	Alcalase 2.4L (Sigma)	>99.5%
2	Esperase 8.0L (Novozymes®)	96.0%
3	Savinase 16.0L (Novozymes®)	98.1%

(a) Reactions conducted using 0.93 mmol of **10** in 1.0 mL DMSO, 250 uL enzyme and 3.75 mL of 200 mM pH 8.7 sodium carbonate buffer at 40 °C for 48 h.

During the reaction development phase, variability was observed in the enzymatic resolution with occasional stalling of the reaction. Evaluation of the batches of (±)-amino ester **10** which exhibited stalling revealed an impurity, diphenyl phosphoramidate (DPP Amide, $(PhO)_2PONH_2$), was present at a higher level than in batches which reached complete conversion. Upon further investigation it was found that diphenyl phosphoramidate, an impurity from DPPA, was carrying through the Curtius process into the isolated (±)-amino ester **10**. A kinetic study of (±)-amino ester **10** with varied levels of diphenyl phosphoramidate was performed (Table 5) and revealed that even after additional enzyme charges the reactions with >0.20 w/w% diphenyl phosphoramidate did not reach completion. A review of the literature confirmed that Alcalase

enzyme, a serine protease, can be irreversibly inhibited by covalent bonding of organophosphorous compounds to the serine hydroxyl via an addition-elimination mechanism.[18] This inhibition does not occur with hydrolytically-stable phosphates (i.e. phosphate esters, monophosphate amide, monophosphate acid, or diphosphate acid). The diphenyl phosphoramidate impurity was therefore controlled at (±)-amino ester **10** to avoid enzyme inhibition.

Table 5: Impact of DPP Amide on the Resolution of (±)-Amino Ester **10**



DPP Amide	(1*R*,2*R*)-Amino Ester **22** ee		
(w/w)	30 h	48 h	193 h
0.13%	98.7%	>99.8%	n/a
0.18%	98.4%	99.6%	n/a
0.42%	92.3%	93.3%	97.5%[b]
0.63%	87.1%	87.3%	96.2%[b]

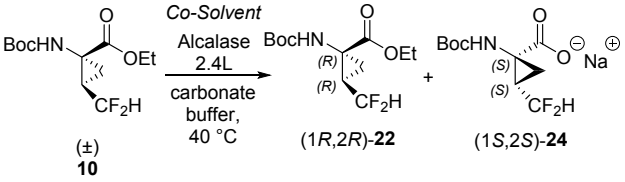
(a) Reactions conducted using 1.79 mmol of **10**, 500 µL Alcalase 2.4L, 1.0 mL of acetone and 9.2 mL of 200 mM pH 8.7 sodium carbonate buffer at 40 °C.

(b) Additional charges of Alcalase 2.4L to 1.75 times the starting amount.

The resolution with Alcalase 2.4L was screened to identify the preferred co-solvent and the co-solvent concentration. A study was performed to screen a variety of organic solvents to identify a co-solvent that could increase substrate solubility without deactivating the enzyme, hindering recovery or impacting downstream clearance (Table 6). Acetone, DMF, and 1,4-dioxane were identified as potential candidate co-solvents due to aiding conversion without affecting selectivity. Based on the high selectivity, conversion rate, low impact to product recovery, and ease of removal in downstream processing, acetone was ultimately chosen as the preferred co-solvent. Next, we turned our attention to the identifying the preferred acetone

content, evaluating from 5% to 30% acetone by volume with respect to total reaction volume. Acetone increased substrate solubility and correspondingly the mass transfer and rate of conversion, but high concentrations had the potential to deactivate the enzyme. As shown in Table 6, acetone at 10% by volume gave the highest rate of conversion and was therefore selected for the process. It was gratifying to note that enzymatic resolution conditions demonstrated in the laboratory were readily scaled to >100 kg to give (1*R*,2*R*)-amino ester **22** in an average 45% assay yield and >99% ee.

Table 6: Co-Solvent Screen for Resolution of (±)-Amino Ester **10**



Co-Solvent	Co-Solvent by Vol	(1*R*,2*R*)-Amino Ester **22** ee 20 h	40 h
IPA	30% [a]	28.6%	38.0%
DMF	30% [a]	96.2%	96.0%
1,4-Dioxane	30% [a]	82.6%	>99.5%
EtOH	30% [a]	8.6%	15.6%
Acetone	30% [a]	83.5%	>99.5%
Acetone	5% [b]	92.9%	>99.5%
Acetone	10% [b]	>99.5%	>99.5%
Acetone	15% [b]	89.4%	>99.5%
Acetone	20% [b]	88.8%	>99.5%
Acetone	30% [b]	68.3%	93.0%

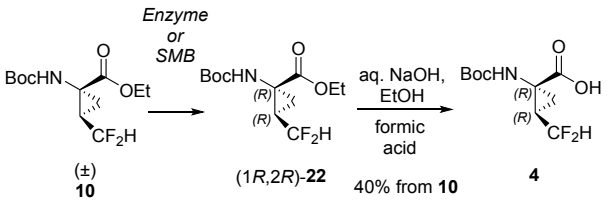
(a) Reactions conducted using 0.18 mmol of **10**, 50 µL Alcalase 2.4L, 0.3 mL of the co-solvent and 0.7 mL of 200 mM pH 8.7 sodium carbonate buffer. (b) Reactions conducted using 0.36 mmol of **10**, 100 µL Alcalase 2.4L, at 1.9 mL total volume of acetone and 200 mM pH 8.7 sodium carbonate buffer.

Completion of the Synthesis of Amino Acid 4

As shown in Scheme 7, after either enzymatic resolution or SMB resolution, the saponification of (1*R*,2*R*)-amino ester **22** was performed in ethanol using sodium hydroxide. The product was

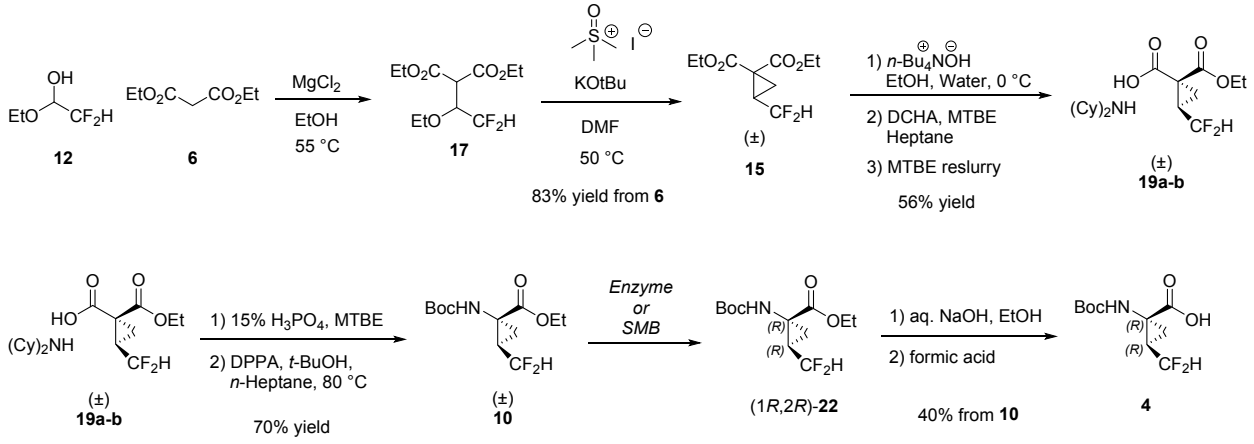
crystallized from the reaction mixture after pH adjustment with formic acid. Amino acid **4** is slightly soluble in water so to improve recovery the ionic strength of the crystallization solution was increased by the addition of sodium chloride. After the development of the crystallization conditions, the (1*R*,2*R*)-Boc-amino acid **4** was isolated in 40% yield for the two-step sequence with >99.5% chiral purity.

Scheme 7: Saponification to (1*R*,2*R*)-Boc-Amino Acid **4**



At only 6 steps and 13% overall yield, the new route to amino acid **4** outlined in Scheme 8 represents a significant improvement over the 10 steps and 7% overall yield of the previously reported enabling route.[1] The enabling route involved a low-yielding fluorination step that utilized DAST to install the difluoromethyl functionality. The new route eliminated the fluorination step by constructing amino acid **4** from the commercially available difluoroacetaldehyde ethyl hemiacetal **12**. With an efficient and scalable route to amino acid **4** in hand, the large-scale production of the sidechain and ultimately glecaprevir was enabled.

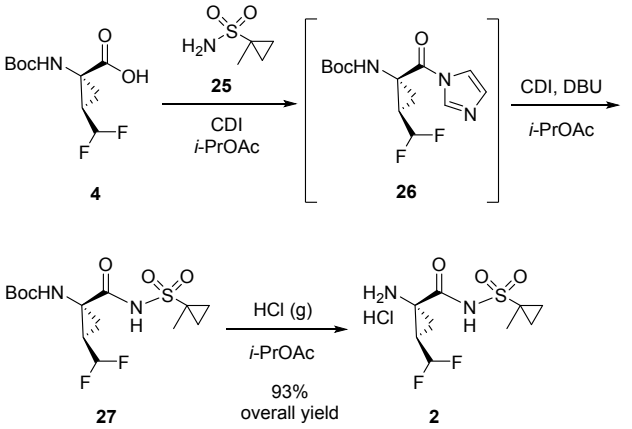
Scheme 8: Overall Synthesis of (1*R*,2*R*)-Boc-Amino Acid **4**



Completion of the Sidechain

The coupling of amino acid **4** with sulfonamide **25**, shown in Scheme 9, was previously accomplished using HATU and DMAP. Four equivalents of DMAP were needed in order to achieve reaction completion in less than 24 h, and the removal of DMAP required one filtration and eight aqueous washes. The deprotection of the Boc group was previously accomplished using 3.5 equivalents of HCl in IPA, with *i*-PrOAc as the main solvent, producing the amino sulfonamide **2** in 89% yield for the two-step sequence. The main goal for future production was to reduce the cycle time of the coupling reaction by finding conditions that would not require multiple steps to remove any reagent or coupling byproducts.

Scheme 9: Synthesis of Sulfonamide **2**



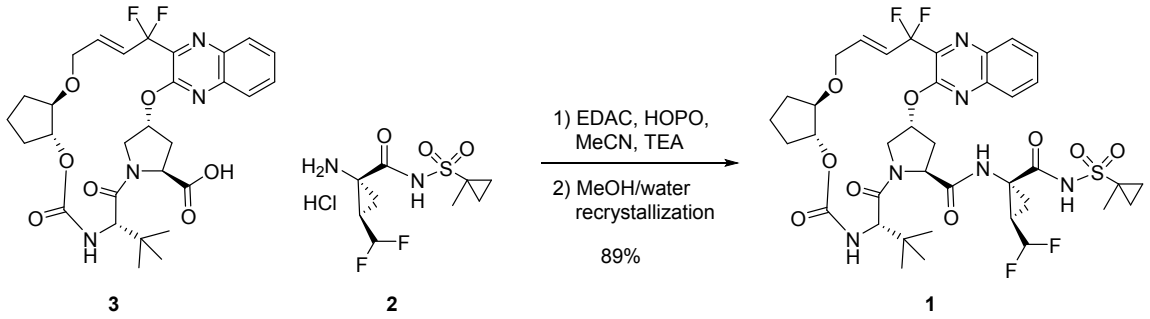
This goal was accomplished by switching to CDI and DBU for the coupling reaction, as used in the synthesis of ABT-450.[19] Amino acid **4** was activated with CDI to form the acyl imidazolide **26** in the presence of sulfonamide **25**, as shown in Scheme 9. The sulfonamide **25** does not react with the acyl imidazolide **26** to a significant extent until the addition of DBU. In order to prevent hydrolysis of the acyl imidazolide, special precautions were implemented to ensure the reactors were free of water. Adventitious water was removed from the reactors by refluxing solvent and drying with nitrogen, and the DBU solution was dried with CDI.[19] The precautions to dry the reaction resulted in the coupling proceeding to greater than 99% conversion. The workup was dramatically simplified relative to the HATU/DMAP process requiring only three aqueous washes to remove the reagent byproducts. Finally, switching to HCl gas for the deprotection eliminated the need to prepare an HCl solution in IPA. These modifications resulted in an increase of the two-step yield of amino sulfonamide **2** to 93%.

Final Assembly of Glecaprevir

As described previously and shown in Scheme 10, the final coupling of amino sulfonamide **2** with macrocycle **3** was accomplished using EDAC and HOPO as the coupling reagents.[1] Initially, this process utilized a preformed solution of the HOPO active ester of **3** which was then transferred to the amino sulfonamide **2** followed by triethylamine addition. However, due to the observed sensitivity of the active ester to hydrolysis with adventitious water it was decided to evaluate a more robust ‘one-reactor’ process, where the acid activation was conducted in the presence of amino sulfonamide **2**. It was envisioned that in the absence of a base, amino sulfonamide **2** would not interfere with the acid activation and the coupling would be initiated by triethylamine addition. Indeed, both the ‘one-reactor’ and the ’two-reactor’ processes were found

to achieve similarly high reaction conversions (typically 99%) in the lab and during early scaleup batches.

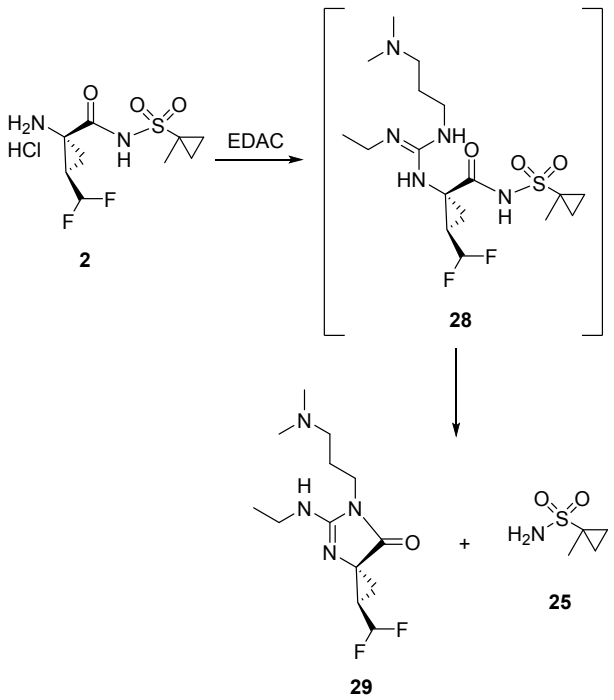
Scheme 10: Final Coupling to form Glecaprevir



3 2 1

However, as more batches were prepared, occasional reaction stalling was observed at 96% conversion of macrocycle **3**. It was subsequently found that even in the absence of base sulfonamide **2** can react with EDAC, resulting in the formation of spirocyclic adduct **29** shown in Scheme 11. This side reaction reduced the amount of amino sulfonamide **2** available for the desired coupling and resulted in the observed stalling.

Scheme 11: Side Reaction of Sulfonamide **2** with EDAC



2 28 29 25

Considering the relatively high rate of this side reaction (~ 25% conversion of **2** observed after 15 min at RT) it was not clear why this had only a minor effect on the actual coupling process. It turned out that the rate of the side reaction was significantly reduced in the presence of HOPO (10% conversion after 15 min at RT). Furthermore, it was noticed that the dissolution of HOPO in acetonitrile was visually slower than dissolution of EDAC and amino sulfonamide **2**. Consequently, the conversion of **2** into **29** decreased further to just 4% when it was subjected to reaction with a preformed solution of EDAC and HOPO. Although there is no evidence of any stable intermediate formation between EDAC and HOPO, it is reasonable to suggest that EDAC can be deactivated via complexation with HOPO and suppress its reaction with **2**.[20]

After this discovery the coupling process was modified to include the preparation of a solution of EDAC and HOPO which was then added to the macrocycle **3** and amino sulfonamide **2** for the acid activation step. Subsequent addition of triethylamine facilitated the coupling. This modified process resulted in reaction conversions consistently above 99% with only 1.07 equivalent of amino sulfonamide **2**. The crystallization of **1** was conducted by addition of aqueous acetic acid as previously described, resulting in an isolated yield of 95%.[1]

Recrystallization of Glecaprevir

Glecaprevir was found to exist in more than 30 different crystalline forms and it exhibited a high propensity to form solvates with various organic solvents. An acetonitrile solvate was isolated from the reaction to form glecaprevir, and subsequently partially de-solvated during drying to remove the acetonitrile to an acceptable level.

Glecaprevir was then recrystallized from a methanol-water solution and isolated in an agitated filter dryer using humidified drying to afford glecaprevir in >94% yield. The process involved

complete dissolution of glecaprevir in methanol at 60 °C. The solution was then cooled to 50 °C followed by addition of a seed slurry in a methanol-water mixture to induce crystallization. The majority of the crystallization was then completed by the slow addition of water to afford crystals with a D_v90 of approximately 400 microns. Wet milling was carried out to reduce the particle size to a desired level. The slurry was then heated to 60 °C to dissolve the fines, and then cooled down to 15 °C to complete the crystallization and produce a methanol/water mixed solvate. The mixed solvate obtained from the crystallization was then isolated in an agitated filter dryer and converted to a hydrate form, using either a series or parallel drying process. The series drying process involved removal of methanol and water under vacuum using heat to produce an anhydrate followed by the rehydration with humidified nitrogen to obtain the desired hydrate form. The parallel drying process incurred concomitant removal of methanol and hydration using humidified nitrogen under vacuum at high temperature to convert the mixed solvate to the desired hydrate.[21]

Conclusion

An efficient process was developed for (1*R*,2*R*)-amino acid **4**, a key intermediate for manufacture of glecaprevir drug substance. The process consists of a unique approach to the construction of the difluoromethyl substituted cyclopropane ring utilizing the commercially available hemiacetal **12**, a diastereoselective saponification of (±)-diester **15** with removal of the minor diastereomer by crystallization, two methods for resolution of (±)-amino ester **10** (enzymatic and SMB chromatography), and a final hydrolysis to synthesize (1*R*,2*R*)-amino acid **4** with chiral purity of >99%. At 6 steps and 13% overall yield, this route to (1*R*,2*R*)-amino acid **4** represents a significant improvement over the 10 steps and 7% overall yield for the enabling route to **4**.[1] This route to **4** also represents a significant improvement in robustness, eliminating

the challenging fluorination chemistry of the enabling route. Combining the new synthesis of **4** with the improvements in the synthesis of amino sulfonamide **2** and the final coupling to form glecaprevir, the overall yield to glecaprevir increased to 11%; double the yield of the enabling route.

Experimental Section

General Information: All reagents and solvents were purchased from commercial vendors and used without further purification. ^{1}H NMR spectra were recorded on a 400 MHz spectrometer, and chemical shifts (δ) are referenced to either TMS or the NMR solvent. ^{13}C NMR spectra were obtained at 101 MHz and referenced to the NMR solvent. Purity results reported by HPLC and GC analysis are listed in area percent.

HPLC samples were analyzed using an Agilent 1200 system equipped with a UV-DAD detector. Numerous HPLC methods were developed for the analysis of the reactions and products. The HPLC columns were typically an Ascentis Express C8 or C18 column (or equivalent), 10 or 15 cm × 4.6 mm, and 2.7 μm particle size. The mobile phases were acetonitrile and either 0.1% H_3PO_4 or 0.1% $HClO_4$ with a gradient from 10% acetonitrile to 90% acetonitrile over 10 to 15 minutes. LC-MS samples were analyzed using similar methods with the exceptions of using 0.1% formic acid or 0.1% trifluoroacetic acid in both the aqueous and the acetonitrile mobile phases, and an ESI detector in positive ion mode.

Analysis by GC was conducted on an HP-5 column, 30 m length × 0.32 mm ID × 0.25 μm film thickness with helium carrier gas and a column flow rate of 1.9 mL/min. The injection temperature was 250 °C, the initial column temperature was 50 °C with a 2 min hold, then a 20 °C/min ramp rate to 260 °C and a 10 min hold at 260 °C. The retention times are **6** at 6.8 min, **16** at 7.7 min, **15** at 8.5 min, **18** at 8.6 min, and **17** at 9.0 min. The chiral GC method for amino ester

22 utilized a Supelco Astec Chiraldex B-DM, 30 m × 0.25 mm, 0.12 μm film thickness column, helium carrier gas, split 1:5, 1.2 mL/min, column temperature at 120 °C hold for 0.5 min, ramp at 10 °C/min to 175 °C, hold for 3 min, ramp at 10 °C/min to 195 °C, hold for 5 min, with an FID. The retention time for **22** is 7.9 min, and 7.5 min for **23**. The chiral HPLC for amino acid **4** utilized a Chiralcel OJ-3R, 150 x 4.6 mm, 3 μm column, 35 °C, mobile phase A: 0.1% phosphoric acid in water, B: MeCN, isocratic: 77:23 ratio of A:B, Flow Rate: 0.5 mL/min UV 205 nm for 17 min. The retention time for **4** is 8.8 min; and 10.1 min for **24**.

Preparation of (±)-Diester 15 via Knoevenagel Condensation and Cyclopropanation: A solution of anhydrous magnesium chloride (0.05 equiv) in anhydrous ethanol (2.3 kg/kg **6**) was prepared under nitrogen. Then difluoroacetaldehyde ethyl hemiacetal (1.1 equiv), diethyl malonate (**6**, 1.0 equiv) and a small ethanol rinse were charged under nitrogen. The mixture was heated to 55 °C and the reaction progress was monitored by GC. After 10 h the reaction was deemed complete, the mixture was cooled to 45 °C and distilled under vacuum. Solvent was exchanged with MTBE and the MTBE solution was filtered and the distillation continued to near completion afford a mixture with 93% purity (combined GC area % for **16** and **17**). The MTBE solution containing mixture of **16-17** was used directly in the next step.

Anhydrous DMF (3.8 kg/kg **6**) was added to the above prepared MTBE solution containing the mixture of **16-17**, purged with nitrogen and heated to 50-55 °C. To a second reactor was charged anhydrous DMF (3.8 kg/kg **6**) and heated to 50-55 °C. To a third reactor was charged anhydrous DMF (7.1 kg/kg **6**), trimethylsulfoxonium iodide (1.3 equiv) and then potassium *tert*-butoxide (1.2 equiv) maintaining the temperature at 10 to 20 °C, mixing until a solution was formed. The solutions in the first and third reactor were charged to the second reactor over approximately 9 h while maintaining the temperature at 50-55 °C, followed by a small rinse of each reactor with

anhydrous DMF. After addition was complete, heating was continued at 50-55 °C and progress of the reaction was monitored by GC. After the reaction was deemed complete, the mixture was cooled to 20 °C, diluted with MTBE (5.9 kg/kg **6**) and quenched with water (8 kg/kg **6**). The layers were separated, and the aqueous layer was re-extracted with MTBE (2 × 5.9 kg/kg **6**). The combined MTBE extracts were washed with 20% brine (8 kg/kg **6**). The MTBE solution was distilled under vacuum to near completion afford the (±)-diester **15** solution (93% purity by GC) for an 83.2% yield for the two steps. A sample was purified by vacuum distillation for characterization. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.68 (tdd, *J* = 55.9, 5.3, 1.7 Hz, 1H), 4.29 – 4.10 (m, 4H), 2.38 – 2.23 (m, 1H), 1.71 (tdd, *J* = 6.2, 2.7, 1.3 Hz, 1H), 1.50 (dddd, *J* = 9.6, 5.4, 2.6, 1.7 Hz, 1H), 1.25 (tdd, *J* = 7.1, 3.9, 1.8 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 168.43, 166.78, 114.62 (dd, *J* = 239.3, 237.7 Hz), 62.24, 62.17, 32.43 (dd, *J* = 5.2, 2.8 Hz), 28.02 (t, *J* = 30.9 Hz), 15.84 (dd, *J* = 6.1, 3.0 Hz), 14.01, 13.97. HRMS calcd $C_{10}H_{15}F_2O_4$ $[M+H]^+$: 237.0933, Found 237.0923.

Preparation of (±)-Monoacid DCHA Salt 19a-b: To a reactor was charged ethanol (4 kg/kg **15**), water (4 kg/kg **15**) and (±)-diester **15** solution (1.0 equiv). The mixture was cooled to 0-5 °C and 40% aqueous tetra-*n*-butyl ammonium hydroxide solution (1.05 equiv) was added slowly over NLT 4 h, rinsing with water (1 kg/kg **15**). The reaction was monitored by HPLC and after 14.5 h was deemed complete. The mixture was diluted with MTBE (7.4 kg/kg **15**) and the pH of the mixture was adjusted to approximately 3 using 3.4% hydrochloric acid. The mixture was warmed to approximately 20 °C and the layers separated, and the aqueous layer was re-extracted with MTBE (3.2 kg/kg **15**). The combined MTBE extracts were washed with 20% brine (4.3 kg/kg **15**) and then water (4.3 kg/kg **15**), and the MTBE solution was distilled under vacuum, chasing with MTBE to dry the solution. The mixture was diluted with MTBE (to 9 L/kg **15**) and

then dicyclohexylamine (1.1 equiv) was added over approximately 2 h followed by addition of *n*-heptane (4.4 kg/kg **15**) over approximately 4 h. The mixture was heated to about 55 °C and refluxed for approximately 1 h and then slowly cooled to 20 °C and aged for approximately 15 h. The slurry was filtered, and the cake was washed with MTBE (1.5 kg/kg **15**). The resulting wetcake and MTBE (4.3 kg/kg **15**) were charged to a reactor and the mixture was heated to about 55 °C and refluxed for 1-2 h and slowly cooled to 20 °C and aged for approximately 15 h. The slurry was filtered, and the cake was washed with MTBE (1.5 kg/kg **15**). The reslurry in MTBE was repeated a second time. The wetcake was dried at 30 °C for 17 h to afford the (±)-monoacid DCHA salt **19a-b** (HPLC purity 99.2%, 0.8% of **19c-d**) in 56.1% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 2H), 5.72 (td, J = 56.2, 5.5 Hz, 1H), 4.15 – 3.92 (m, 2H), 2.97 (tt, J = 10.8, 3.7 Hz, 2H), 2.03 – 1.93 (m, 4H), 1.92 – 1.82 (m, 1H), 1.81 – 1.64 (m, 4H), 1.59 (dt, J = 13.0, 3.2 Hz, 2H), 1.39 – 1.18 (m, 8H), 1.15 (t, J = 7.1 Hz, 4H), 1.12 – 0.98 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.52, 169.08, 116.44 (t, J = 235.7 Hz), 59.88, 51.66, 34.70 (d, J = 3.4 Hz), 28.64, 25.47 (dd, J = 29.7, 27.8 Hz), 24.92, 24.08, 13.89, 12.34. HRMS calcd $C_8H_{10}F_2NaO_4$ $[M+Na]^+$: 231.0439, Found 231.0444. Melting point 152-154 °C.

Preparative HPLC Separation of Monoacid Isomers 14a-d for Determination of Stereochemistry: The first preparative HPLC separation utilized a ChiralPak IC (21 mm x 250 mm, and 5 micron) column, and isocratic elution over 40 min with the mobile phase 98 vol % *n*-heptane, 2 vol % isopropanol, and 0.1 vol % trifluoroacetic acid, at a flow rate of 30 mL/min and detection at 215 nm. A solution of monoacid **14a-d** was prepared in the mobile phase at 25 mg/mL concentration. Injections of 5-6.5 mL were separated and allowed isolation of **14a** and **14d** as individual compounds, and **14b** and **14c** as a mixture. The mixture of **14b** and **14c** was purified by a second preparative HPLC separation utilizing an (*S*,*S*)-Whelk-O1 5/100 Kromasil

(30 mm x 250 mm, and 5 micron) column, the same mobile phase as described above, and an isocratic elution over 20 minutes at 35 mL/min with detection at 230 nm. The mixture of **14b** and **14c** was dissolved dichloromethane (22 mg/mL) and purified in 2 mL injections to isolate **14b** and **14c** as individual compounds.

Conversion of Monoacids 14a-d to DCHA Salts 19a-d for X-Ray Analysis: Monoacid **14a** (1.0 equiv) was dissolved in MTBE (5.7 mL/g **14a**) and dicyclohexylamine (1.1 equiv) and heptanes (11.3 mL/g **14a**) were charged with stirring at ambient temperature. The resulting slurry was mixed overnight, filtered and the wetcake was washed with a mixture of 1:2 v/v MTBE/heptanes solution and dried at 45 °C under vacuum to give the DCHA salt **19a** as a solid in 30% yield (purity: 97.1%, ee: >99.5%, de: >99.5%). The procedure described above was adapted for the isolation of DCHA salts **19b-d**. The DCHA salt **19b** was obtained in 41% yield (purity: >99.5%, ee: >99.5%, de: 98.6%). The DCHA salt **19c** was obtained in 61% yield (purity: >99.5%, ee: >99.5%, de: 92.6%). The DCHA salt **19d** was obtained in 54% yield (purity: 97.3%, ee: >99.5%, de: >99.5%).

Preparation of (±)-Amino Ester 10 via Curtius Rearrangement: To a reactor was charged MTBE (7.5 kg/kg **19a-b**) and (±)-monoacid DCHA salt **19a-b** (1.0 equiv) and then washed with three times with 20% phosphoric acid (total of 8.8 kg/kg **19a-b**), followed by 20% brine (2 × 2.8 kg/kg **19a-b**). The MTBE solution was distilled under vacuum and exchanged with *n*-heptane. To this mixture, *n*-heptane (3.4 kg/kg **19a-b**), *tert*-butanol (3.8 kg/kg **19a-b**) and triethylamine (1.54 equiv) were added and the mixture heated to 75-85 °C. To this mixture was added diphenylphosphoryl azide (0.99 equiv) over approximately 9 h. Heating was continued for another 9 h and the reaction was deemed complete by HPLC analysis. The mixture was cooled to approximately 25 °C and quenched with water (3.9 kg/kg **19a-b**) and mixed for approximately 5

h and then settled. The aqueous layer was separated and then re-extracted with a mixture of MTBE (0.9 kg/kg **19a-b**) and *n*-heptane (4.6 kg/kg **19a-b**). The combined organic layers were washed sequentially with 5% citric acid (5.5 kg/kg **19a-b**), 7.6% sodium bicarbonate (2.9 kg/kg **19a-b**), and water (2 × 1.5 kg/kg **19a-b**). The organic layer was distilled under vacuum and switched to *n*-heptane. The mixture was diluted with *n*-heptane (to 3.1 L/kg **19a-b**) and heated to dissolve the solids and then cooled to 35 °C and seeded with (±)-amino ester **10**. The mixture was cooled to 0-5 °C over 10 h and aged for 5 h. The resulting slurry was filtered and wetcake was washed with cold *n*-heptane (0.5 kg/kg **19a-b**). The wetcake was dried at 25-30 °C under vacuum for 15 h to afford (±)-amino ester **10** (HPLC purity 98.4%, 98.6% assay) in 69.6% yield. Rotational isomers were observed in the NMR spectra. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 – 7.36 (m, 1H), 6.12 – 5.66 (m, 1H), 4.23 – 4.01 (m, 2H), 2.02 – 1.88 (m, 1H), 1.77 – 1.62 (m, 1H), 1.38 (s, 10H), 1.26 – 1.12 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.45, 155.42, 116.06 (dd, J = 236.6, 233.0 Hz), 78.45, 61.27, 37.10 (d, J = 9.3 Hz), 30.22 (dd, J = 33.8, 30.3 Hz), 28.06, 19.19 (d, J = 8.1 Hz), 13.87. HRMS calcd $C_{12}H_{19}F_2NNaO_4$ $[M+Na]^+$: 302.1174, Found 302.1176. Melting point 66-67 °C.

Resolution of (±)-amino ester 10 via Simulated Moving Bed Chromatography: A solution of (±)-amino ester **10** was dissolved in acetonitrile at 53 g/L concentration. The feed solution was resolved using Chiralpak AY (Particle Size: 20 μm) as the stationary phase and 8 × 200 mm diameter columns with a 90 mm bed length. The (1*R*,2*R*)-amino ester **22** was isolated as an acetonitrile solution (99.0% ee) in 41-47% yield.

Enzymatic Resolution (±)-amino ester 10: A 1.67% sodium bicarbonate solution (17.5 kg/kg **10**) was diluted with a 2.0% sodium carbonate solution (0.5 kg/kg **10**) to adjust the pH to 8.7. The mixture was heated to 40 °C and a solution of (±)-amino ester **10** (1.0 equiv) in acetone was

charged slowly followed by an acetone rinse (total of 1.6 kg/kg **10**). Alcalase 2.4L (1.2 kg/kg **10**) was then added and the mixture was stirred at 40 °C until the (1*S*,2*S*)-amino ester **23** was reduced below 0.5% by GC analysis. The mixture was cooled to 20 °C and then MTBE (7.4 kg/kg **10**) was added, and the layers separated. The aqueous layer was re-extracted with MTBE (3.7 kg/kg **10**) and the combined MTBE extracts were washed with 4.8% sodium bicarbonate (5.3 kg/kg **10**), followed by water (5.0 kg/kg **10**). The MTBE solution was distilled under vacuum to afford (1*R*,2*R*)-amino ester **22** as an MTBE solution (99.6% ee) in 45% yield.

Saponification to (1*R*,2*R*)-Amino Acid 4: (1*R*,2*R*)-amino ester **22** as an MTBE or acetonitrile solution (1.0 equiv) was further concentrated under vacuum and exchanged with ethanol (final volume of 2.4 L/kg **22**). The mixture was adjusted to 20 °C and a 10.4% sodium hydroxide solution (1.5 equiv) was charged and mixed for approximately 7.5 h until the reaction was complete by HPLC analysis. The reaction was quenched with formic acid (2.14 equiv) and heated to approximately 30 °C. A slurry of (1*R*,2*R*)-amino acid **4** seed (0.03 kg/kg **22**) in water (2.9 kg/kg **22**) and ethanol (0.08 kg/kg **22**) was then added over approximately 4 h while maintaining the temperature at 30 °C. Then a 20% brine solution (3 kg/kg **22**) was added slowly. The resulting slurry was cooled to 0-5 °C, filtered, and the wetcake was washed with water (2 kg/kg **22**). The wet cake was dried at 45-50 °C under vacuum for NLT 10 h to afford (1*R*,2*R*)-amino acid **4** (99.7% assay, 99.7% ee) in 40.3% yield over the two-step sequence. Spectral data was identical to that previously reported in the literature.[1]

Amino Sulfonamide 2: The (1*R*,2*R*)-amino acid **4** (1.0 equiv), sulfonamide **25** (1.1 equiv), and CDI (1.1 equiv) were charged to a reactor followed by *i*-PrOAc (4.5 kg/kg **4**). The solution was mixed at 20 °C until HPLC analysis showed complete consumption of starting material.[22] In a separate reactor was charged CDI (0.05 equiv), *i*-PrOAc (1.9 kg/kg **4**) and DBU (1.6 equiv).

The DBU solution was mixed for 30 min. at 25 °C then transferred to the acyl imidazolide. The reaction was mixed at 25 °C for 4 h and HPLC analysis showed the acyl imidazolide was consumed.[22] The reaction was diluted with *i*-PrOAc (14.8 kg/kg **4**) and warmed to 40 °C. The solution was then washed with 20% phosphoric acid (9.7 kg/kg **4**), and then water (2 × 8.1 kg/kg **4**). After each wash, *i*-PrOAc was added as needed to maintain the original volume. The filtrate was concentrated by vacuum distillation, charging additional *i*-PrOAc to maintain the volume (14 L/kg **4**) and dry the solution. Towards the end of the distillation Boc sulfonamide **27** crystallizes out of solution. The resulting slurry was cooled to 0 °C and HCl gas (5.9 equiv) was charged into the slurry maintaining an internal temperature of NMT 15 °C. Upon completion of the HCl addition the slurry was mixed at 30 ± 10 °C for 9 h and HPLC analysis of the supernatant showed reaction completion. The slurry was cooled to 20 ± 5 °C and mixed for 2 h. The solids were filtered, washed with *i*-PrOAc (1.7 kg/kg **4**) and dried under vacuum to give amino sulfonamide **2** (99.6% potency) for a yield of 93% over the two steps. Spectral data was identical to that previously reported in the literature.[1]

Glecaprevir 1: To a reactor was charged macrocycle **3** (1.0 equiv) and amino sulfonamide **2** (1.07 equiv). To another reactor was charged 2-hydroxypyridine *N*-oxide (1.3 equiv), EDAC (1.3 equiv) and MeCN (5.6 kg/kg **3**) and then mixed at approximately 20 °C until the solids dissolved. The solution of EDAC/HOPO was then transferred to the reactor containing **2** and **3**, rinsing with MeCN (0.5 kg/kg **3**) to complete the transfer. After mixing for 1 h the solution was cooled to approximately 10 °C and triethylamine (1.7 equiv) was added and the mixture warmed to approximately 20 °C. After mixing for 16 h, HPLC analysis showed >99% conversion of **3**. The reaction was quenched with a 20.5% acetic acid solution (1 kg/kg **3**), then heated to 45 °C, followed by an addition of water (2 kg/kg **3**). The solution was cooled to 30 °C and then seeded

and mixed until crystallization occurred. To this slurry was then added water over approximately 15 h. The slurry was then cooled to 20 °C, aged for NLT 1 h and then filtered and the product washed with a solution of MeCN (1.4 kg/kg **3**) and water (2.2 kg/kg **3**). The wet cake was blown dry on the filter with nitrogen and then dried under vacuum with heating to afford the initial product in 95% yield. The initially isolated product was recrystallized with the modifications described earlier, and consistent with the previously described procedure.[1] The average overall yield through the initial isolation and recrystallization was 91%. Spectral data was identical to that previously reported in the literature.[1]

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DISCLOSURES

AbbVie and Enanta sponsored and funded the study, contributed to the design, participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication. All authors are or were employees of AbbVie and may own AbbVie stock. Chen Ding is currently an employee of Alnylam Pharmaceuticals, Inc.

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SUPPORTING INFORMATION

NMR spectra for compounds **10**, **15**, **19a-b**, and the EDAC/HOPO mixture; crystal structure data for compounds **19a**, **19b**, and **19d**.

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[20] After addition of EDAC to a solution of HOPO in CD_3CN, analysis by ^1H NMR shows a new, second set of HOPO related signals in a ratio of 0.85:1. See the Supporting Information for the spectra.
[21] A complete discussion of the crystallization and drying process design to manufacture glecaprevir with the desired physical properties will be published in a separate communication.
[22] HPLC samples were quenched into 1.5% DBU in methanol to allow quantification of the acylimidazolide as the corresponding methyl ester.