Identifying a diastereomeric salt for a challenging chiral resolution

Advancing a therapeutic for malaria using the freeslate system

Researchers at the Medicines for Malaria Venture were pursuing a promising candidate for the treatment of malaria when they ran into a roadblock. For over a year they attempted to develop a more direct method for isolating a key chiral intermediate. Until this challenge was overcome, the program was stalled and could not progress. Leveraging the power of Unchained Labs' freeslate system configured for preformulation, they overcame this roadblock by identifying and scaling-up a viable chiral resolution method in just two weeks.

The Medicines for Malaria Venture

Worldwide, 3.3 billion people are at risk for malaria, which is prevalent in tropical and subtropical countries. According to the Centers for Disease Control, in 2010 an estimated 219 million cases of malaria occurred worldwide and 660,000 people died, 91% of whom lived in Africa. Globally, 86% of those who died from malaria were children. Malaria is a disease caused by several species of parasites in the genus Plasmodium. These parasites are carried by infected Anopheles mosquitoes that feed on humans. When diagnosed early, malaria is a preventable and treatable disease; however, the emergence of parasites that are resistant to anti-malarial drugs has compounded the problem of malaria infection. In 1999, a group from the World Health Organization formed the Medicines for Malaria Venture (MMV), a not-for-profit public partnership.

MMV used Unchained Lab's high-throughput techniques to deliver successful results in approximately two weeks to a problem that they had been trying to solve for more than one year using traditional methods.

The mission of MMV is to reduce the burden of malaria in disease-endemic countries by discovering, developing and facilitating the delivery of new, effective and affordable anti-malarial drugs. They envision a world in which innovative medicines will cure and protect the vulnerable and under-served populations at risk of malaria, and help to ultimately eradicate this terrible disease. In 1999, there were no compounds in the MMV portfolio. Ten years later, MMV had a robust portfolio consisting of 42 development programs, including four anti-malarial compounds in Phase 3 clinical trials and three compounds in registration—all funded solely by MMV. The MMV portfolio has continued to grow, with new compounds from translational research moving forward and six drugs approved for the treatment of malaria.

Discovering novel chemotypes

MMV identified several potential anti-malarial chemotypes using high-throughput screening. One screen, performed at St. Jude Children's Hospital, identified multiple validated series of hits.[1] Of these, a series of SJ557733, such as the compound in Figure 1, had progressed to the lead optimization stage. Studies of this series have identified the chiral carboxylic acid (+)-2 as a key intermediate (Figure 2).

Initial assessments by MMV to determine the viability of using a chiral resolution method for the key carboxylic acid enantiomer proved challenging. Figure 3 shows their original route used to prepare the desired chiral carboxylic acid (+)-2 on a relatively large scale (30 g). The resolution was achieved via two-step synthesis (esterification and subsequent hydrolysis) and a preparative supercritical fluid chromatography (SFC) chiral separation. This route resulted in good recovery and enantiomeric excess (*ee*), but the two synthetic steps and preparative chiral column purification made the process cumbersome.

Another promising approach was chiral resolution via diastereomeric salt formation (Figure 4) with amino alcohols. This approach used chiral *trans*-1-amino-2-indanol to form the diastereomeric salts and a 1:1 propionitrile (EtCN):methyl tert-butyl ether (MTBE) solvent system to achieve separation based on solubility differences. However, the recovery was poor and insufficient for a large-scale separation.

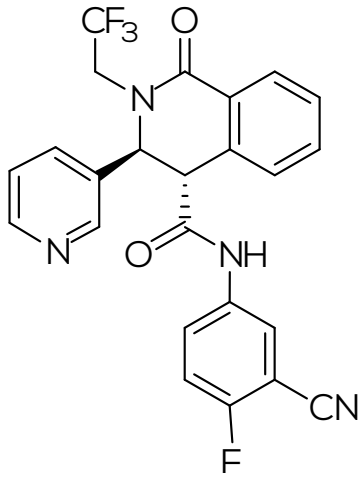


Figure 1: SJ557733.

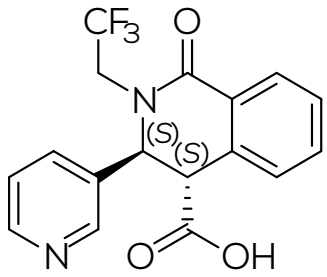


Figure 2: Injection and sampling with controlled atmosphere sampling port technology.

MMV and Unchained Labs' collaboration

To advance development of this novel chemotype, an efficient, facile method to resolve the desired enantiomeric intermediate from its racemate was needed. MMV and Unchained Labs worked together to develop a chiral resolution method to prepare carboxylic acid (+)-2 in excellent yield and *ee*. Using the freeslate system, the team developed the screening strategy, designed the experiments, performed 144 chiral resolution experiments, and analyzed and reported the results (Figure 5).

High-throughput chiral resolution screening

With the prior chiral resolution results in mind, the focus was to gain a better understanding of the solubility of the diastereomeric salts formed, with the goal of isolating the desired (+)-2 salt as a solid. To do this, a screen using chiral amino alcohols (Table 1), including chiral *trans*-1-amino-2-indanol, and 12 different solvents and solvent mixtures (Table 2) was undertaken.

On the first day of screening, the diastereomeric salts of racemic-2 were formed by dispensing 32.5 mg (93 µmol) of racemic-2 into a 96-well microplate using the powder dispense capability of the system. Next, 500 µL of methanol solution containing 93 µmol (1 equivalent) of chiral amino alcohol were added to the rows of the microplate as per Table 1. The plate was sealed and stirred for two hours at 45 °C to encourage diastereomeric salt formation. At the conclusion of the first day, the methanol was removed by evaporation using a Genevac HT-12 Evaporator and stored under high vacuum overnight.

On the second day, 500 µL of the 12 resolution solvents and solvent mixtures were added to the columns of the 96-well microplate as per Table 2. The microplate was sealed for 20 hours under the conditions shown in Figure 6.

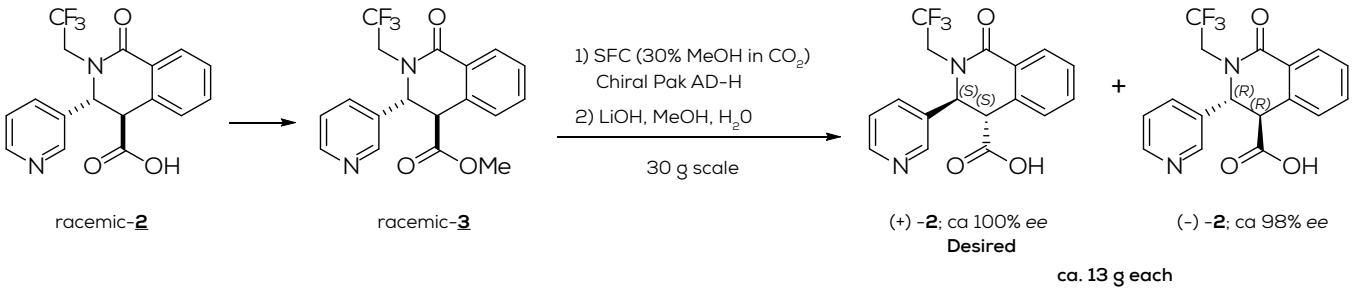


Figure 3: Original large-scale route to obtain (+)-2. SFC: supercritical liquid chromatography, MeOH: methanol.

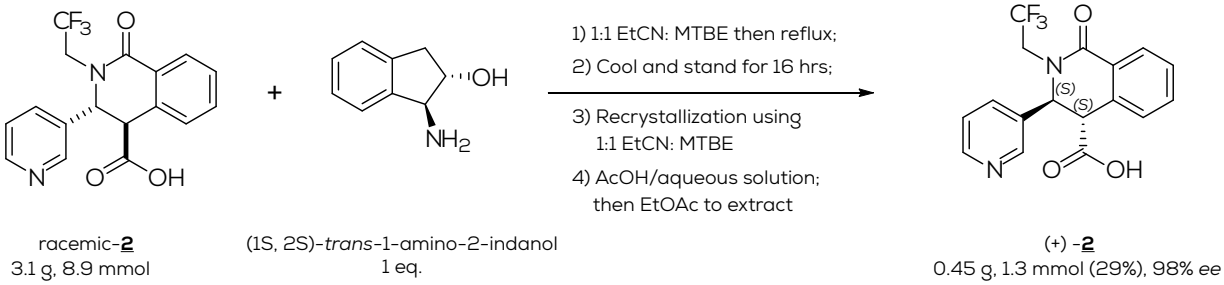


Figure 4: Original chiral resolution using *trans* amino indanol. (EtCN: propanenitrile, MTBE: methyl tert-butyl ether, AcOH: acetic acid, EtOAc: ethyl acetate).

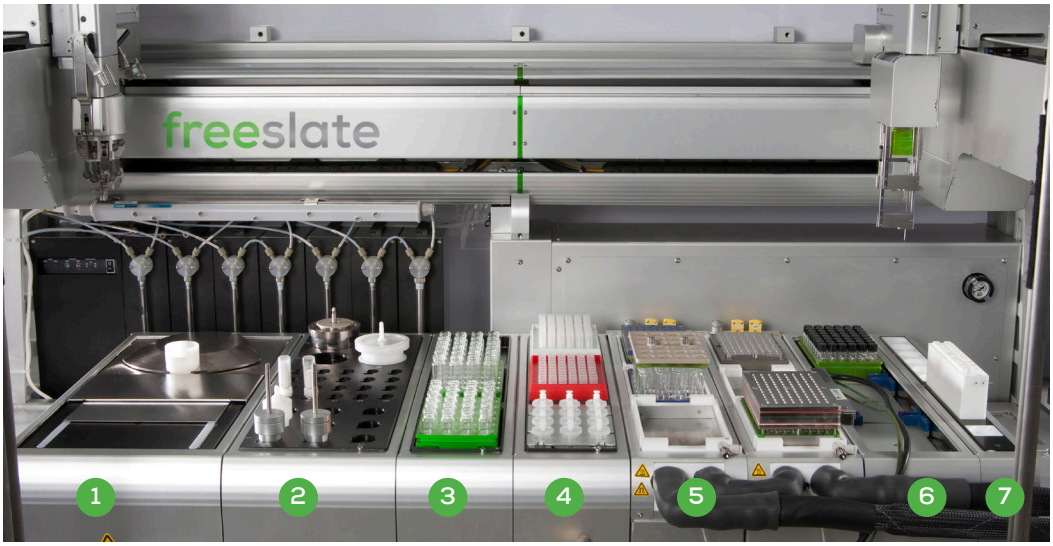


Figure 5: The deck elements of the freeslate system configued for preformulation, (**1**) 5-place balance with integrated camera, (**2**) tip rack holder, (**3**) vortexing station, (**4**) combination rack, (**5**) two heating/cooling/stirring stations – 1 cooled position, (**6**) capping/decapping station, and (**7**) wash station. The arm elements (not shown) include a vial/plate gripper, 1-tip liquid dispenser, heated 4-tip liquid dispenser and flexible tool changer.

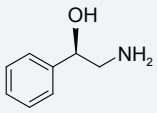
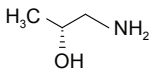
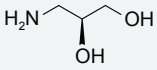
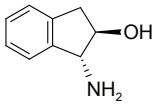
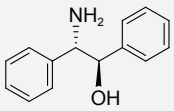
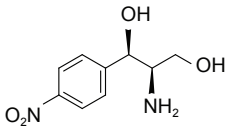
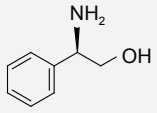
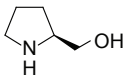
Row	Chemical structure	Amino alcohol
A		(R)-(-)-2-amino-1-phenylethanol
B		(R)-(-)-2-amino-2-propanol
C		(S)-3-amino-1,2-propanediol
D		(1R,2R)-(-)-*trans*-1-amino-2-indanol
E		(1R,2S)-(-)-2-amino-1,2-diphenylethanol
F		(1R,2R)-(-)-2-amino-1-(4-nitrophenyl)-1,3-propanediol
G		(R)-(-)-2-phenylglycinol
H		(S)-(+)-2-pyrrolidinemethanol

Table 1: Chiral amino alcohols for first screen, listed by microplate row.

Column	Solvent / solvent mixture
1	EtCN:MTBE (1:1)
2	Acetone
3	Acetone:H2O (9:1)
4	Acetone:Heptane (1:1)
5	MeOH
6	MeOH:H2O (9:1)
7	IPA
8	IPA:H2O (9:1)
9	THF:H2O (9:1)
10	MeCN
11	MeCN:H2O (9:1)
12	EtOAc

Table 2: Solvents and solvent mixtures for first screen, listed by microplate column. EtCN: propanenitrile, MTBE: methyl tert-butyl ether, MeOH: methanol, IPA: isopropyl alcohol, THF: tetrahydrofuran, MeCN: acetonitrile and EtOAc: ethyl acetate.

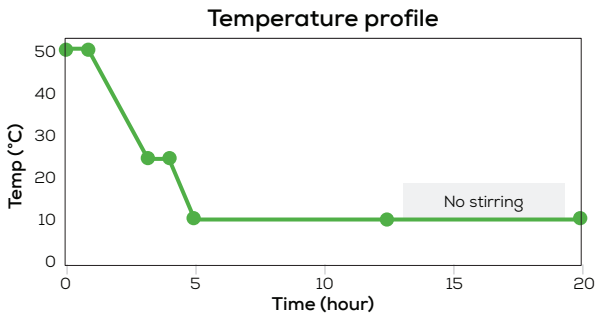
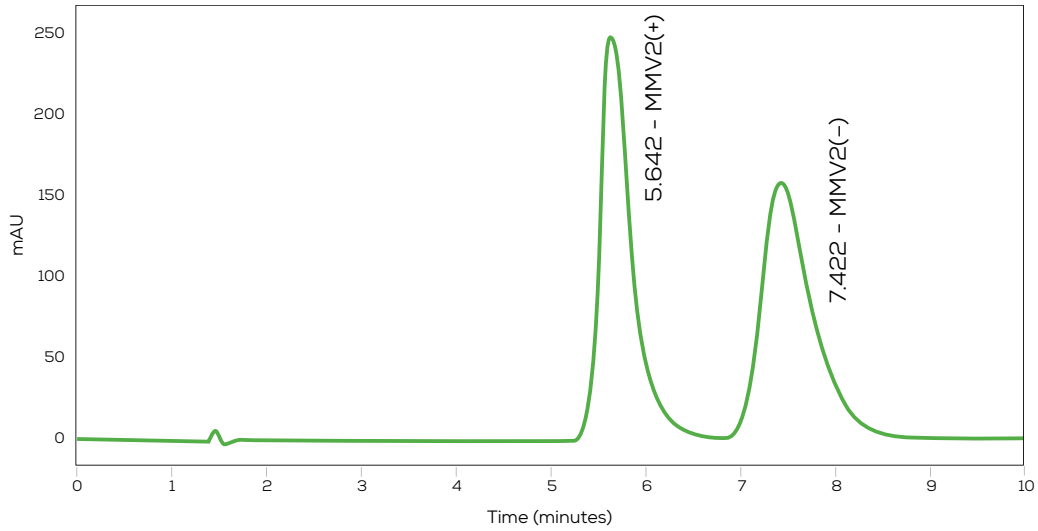


Figure 6: Temperature and stiring conditions for both screens.

Ideally one of the first day formed diastereomers would be fully dissolved while the other would remain as a solid. On the third day, using the heated 4-tip liquid dispenser of the freeslate system, supernatent was transferred to heated filter block. Post filtration, an HPLC plate was prepared by transferring 40 µL of filtrate to a new 96-well microplate, and diluted to an appropriate concentration for analysis. The original wells of the 96-well microplate were visually inspected for solids, and the supernatant from all wells with solid were analyzed via chiral HPLC to assess the difference in the solubility of the diastereomers (Figure 7).

From the first round of screening, only one moderate hit was identified, (1R,2R)-*trans*-1-amino-2-indanol in ethyl acetate (EtOAc), with an overall solution yield of 38% and 72% *ee*.



• **Column:**	Chiralpak AD-H (4.6x150 mm, 5.0 µm)
• **Mobile phase:**	Solvent A: Heptane
	Solvent B: Reagent alcohol with 0.1% TFA
• **Flow rate:**	1.2 mL/min
• **Column temp:**	35 °C
• **Injection volume:**	3 µL
• **Detection:**	210 nm
• **LC duration:**	10 mins +2 min post run
• **Isocratic mobile phase:**	30% Solvent B

Figure 7: Chiral HPLC analysis of racemic-2 standard.

Following up on this moderate hit, a more focused screen was planned, which included all four isomers of 1-amino-2-indanol (Table 3). In addition, the primary screen displayed high solubility within many fully dissolved wells. In order to overcome this issue, the hope was to lower overall solubility by adding 50% Heptane to the affected wells on the secondary screen (Table 4). Previously, MMV conducted a one-off study that indicated 2 eq. of salts might be needed for complete salt formation. Both 1:1 and 2:1 chiral 1-amino-2-indanol to racemic-2 were tested on the secondary screen as well (Table 5).

From the results, three conclusions were clear:

- EtCN:heptane (1:1) was identified as the best solvent mixture
- Only *trans*-1-amino-2-indanols resolved racemic-2
- A 2:1 ratio of *trans*-1-amino-2-indanol:racemic-2 was required to form the diastereomeric salt

The chiral HPLC analysis of the best hit, well A5 is shown in Figure 8. From the HPLC analysis, the (-)-2 salt had a solubility of 27.5 mg/mL and the (+)-2 salt had a solubility of ~0.03 mg/mL. In effect, (-)-2 salt was about 1,000 times more soluble than the (+)-2 salt, and this dramatic difference in solubility provided excellent resolution of the diastereomers.

Scale-up by MMV and Rutgers University

Based on the parameters from the follow-up screen, MMV and Rutgers University scaled-up the experiment to prepare the (+)-2 acid (Table 5). To do this, they suspended 16.4 g of racemic acid (±)-2 and 14 g of (1S,2S)-*trans*-1-amino-2-indanol (2 eq.) in 500 mL of 1:1 EtCN:heptane and refluxed the mixture for 3.5 hours. The solubility of the 2:1 (1S,2S)-1-2-indanol:(+)-2 salt was low enough that the mixture was a slurry, even under reflux

conditions. After 3.5 hours, the solution was cooled to room temperature and filtered. To ensure that all the (-)-2 salt was removed, the solid was washed with an additional 200 mL of 1:1 EtCN:heptane. The scale-up yielded 14.3 g of the 2:1 1-amino-2-indanol:(+)-2 salt (94% theoretical yield) with a 99.3% *ee*. To generate the free (+)-2 acid, the 2:1 1-amino-2-indanol:(+)-2 was washed with 20% acetic acid and then extracted with EtOAc. The EtOAc extracts were combined, dried and concentrated. This method generated 7 g of the (+)-2 acid (85% of theoretical amount) with a 99.3% *ee*.

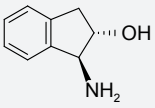
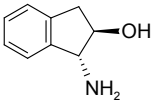
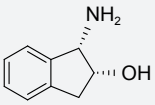
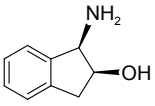
Row	Chemical structure	Amino alcohol
A		(1S, 2S)-*trans*-1-amino-2-indanol
B		(1R, 2R)-*trans*-1-amino-2-indanol
C		(1S, 2R)-*cis*-1-amino-2-indanol
D		(1R, 2S)-*cis*-1-amino-2-indanol

Table 3: Chiral 1-amino-2-indanols for follow-up screen, listed by microplate row.

Summary

The work described in this paper used Unchained Labs' high-throughput techniques to deliver successful results in approximately two weeks to a problem that MMV had been trying to solve for over a year using traditional methods.

During those two weeks, Unchained Labs and MMV developed the screening strategy, designed the experiments, ran two screens (144 experiments) using the freeslate system, performed the chiral HPLC analysis and analyzed the results. In

Solvent ID	Solvent / solvent mixture
S1	EtOAc
S2	BuOAc
S3	EtOAc:Heptane (1:1)
S4	EtOH:Heptane (1:1)
S5	EtCN:Heptane (1:1)
S6	DME:Heptane (1:1)
S7	MIBK:Heptane (1:1)
S8	Me-THF:Heptane (1:1)
S9	Toluene

Table 4: Solvents and solvent mixtures for follow-up screen. EtOAc: ethyl acetate, BuOAc: butyl acetate, EtOH: ethanol, EtCN: propanenitrile, DME: dimethoxyethane, MIBK: methyl isobutyl ketone and Me-THF: 2-methyl tetrahydrafuran.

		Concentrations of 2:1 1-Amino-2-Indanol (+)-2 and (-)-2 salts (mg/mL) in solution											
		1	2	3	4	5	6	7	8	9	10	11	12
		1-Amino-2-Indanol:2 Ratio:2:1									1:1		
		EtOAc	t-BuOAc	EtOAc: Heptane (1:1)	EtOH: Heptane (1:1)	EtCN: Heptane (1:1)	DME: Heptane (1:1)	MIBK: Heptane (1:1)	Me-THF: Heptane (1:1)	Toluene	EtOAc	EtOH: Heptane (1:1)	Toluene
A	(1S, 2S)-*trans*	0.7 (+) 14.6 (–)	0.0 (+) 2.9 (–)	0.0 (+) 0.1 (–)	3.4 (+) 25.2 (–)	0.03 (+) 27.5 (–)	0.0 (+) 3.4 (–)	0.0 (+) 0.3 (–)	1.2 (+) 1.8 (–)	0.0 (+) 0.0 (–)	8.4 (+) 20.9 (–)	26.2 (+) 25.7 (–)	0.0 (+) 0.0 (–)
B	(1R, 2R)-*trans*	24.9 (+) 0.0 (–)	3.2 (+) 0.0 (–)	1.4 (+) 0.0 (–)	25.7 (+) 25.4 (–)	30.9 (+) 0.00 (–)	5.3 (+) ND (–)	0.6 (+) 0.0 (–)	0.9 (+) ND (–)	0.1 (+) 0.0 (–)	27.1 (+) 26.3 (–)	29.8 (+) 29.4 (–)	1.1 (+) 1.0 (–)
C	(1S, 2R)-*cis*		4.4 (+) 4.1 (–)	2.4 (+) 2.2 (–)		38.8 (+) 38.3 (–)	6.1 (+) 5.9 (–)	0.1 (+) 0.0 (–)	1.1 (+) 1.0 (–)	1.1 (+) 1.0 (–)			2.5 (+) 2.4 (–)
D	(1R, 2S)-*cis*		12.2 (+) 11.7 (–)	2.5 (+) 2.2 (–)		43.3 (+) 42.7 (–)	3.0 (+) 3.0 (–)	0.9 (+) 0.8 (–)	2.6 (+) 2.0 (–)	7.9 (+) 7.3 (–)			ND (+) ND (–)

Table 5: Results of follow-up screen. EtOAc: ethyl acetate, BuOAc: butyl acetate, EtOH: ethanol, EtCN: propanenitrile, DME: dimethoxyethane, MIBK: methyl isobutyl ketone and Me-THF: 2-methyl tetrahydrafuran.

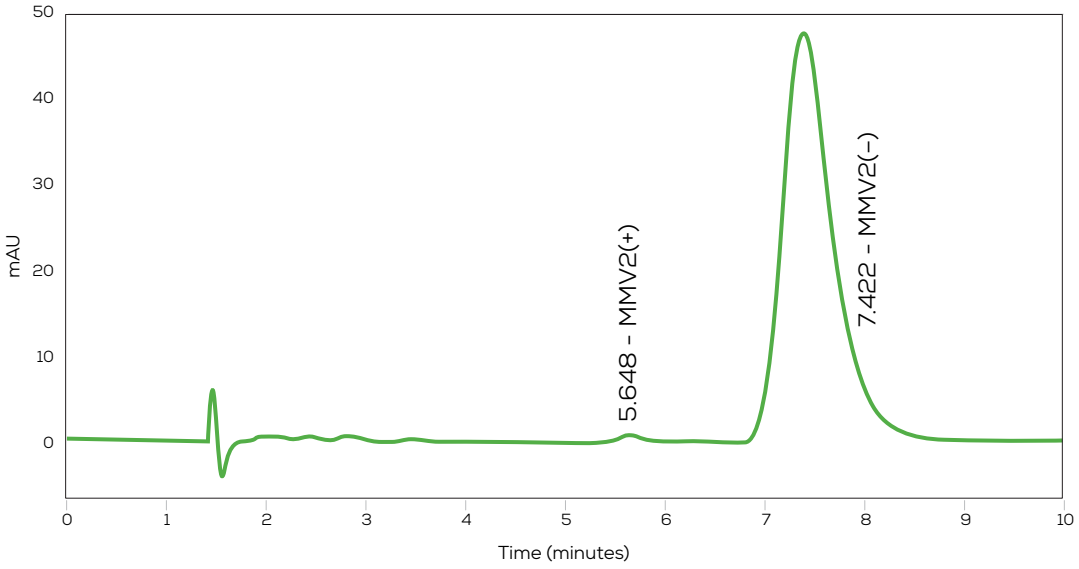


Figure 8: Chiral HPLC analysis of well A5 from the follow-up screen.

total, 11 chiral amino alcohols, 20 solvents and solvent mixtures and two equivalent-levels of counterions were screened. This study identified a chiral resolution condition to isolate a key (+)-2 intermediate with both excellent yield and *ee*. This condition discovered via high-throughput screening was also amenable to scale-up, which carboxylic acid (+)-2 with 85% yield and 99.3% *ee*. Until the challenge of purifying this key intermediate was overcome, MMV's development program was unable to progress. As a result of this work, MMV has begun using this method to prepare the desired (+)-2 carboxylic acid, which enabled the advancement of their promising anti-malaria drug candidate, SJ557733.

References

1 Guiguemde, W. A. et al. Chemical genetics of *Plasmodium falciparum*. *Nature*. 2010; 465; 311–315.

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