Protecting Group-Free Synthesis of the Antimalaria Drug MMV693183

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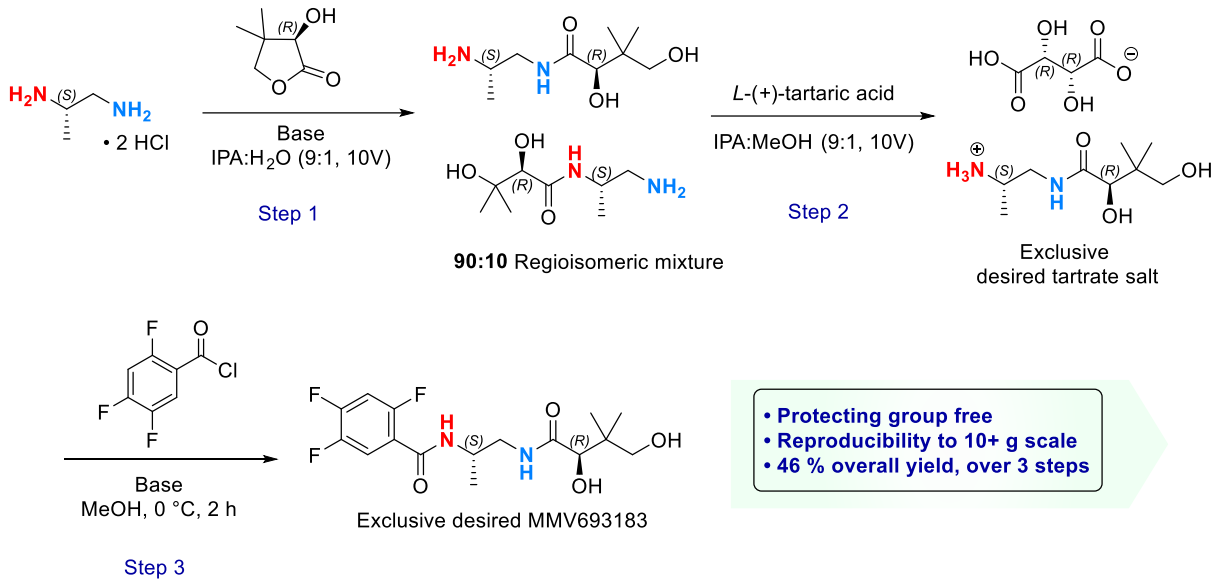


KEYWORDS

MMV693183, antimalaria, regioselective synthesis, protecting group free, API

ABSTRACT

MMV693183 is a promising antimalarial drug candidate that works for uncomplicated malaria treatment and resistance management. Herein, we report an efficient and highly regioselective synthesis of MMV693183. This efficient approach to MMV693183 is only three

steps from readily available starting materials and provides the API in 46 % overall yield without requiring protecting groups.

INTRODUCTION

Malaria remains one of the most devastating parasitic diseases causing more than 241 million cases and 627 thousand estimated malaria deaths in 2020 according to the World Health Organization (WHO).[1] The resistance to current antimalarial drugs and the high costs of treatment demands the search for new therapeutic agents.[2–5] Pantothenic acid (vitamin B5) (Figure 1) is one of the important precursors of the essential enzyme cofactor coenzyme A (CoA) on which the predominant pathogen for Malaria *Plasmodium falciparum* is dependent during the intraerythrocytic stage of its life cycle.[6] In the last few decades many analogues of pantothenic acid have been synthesized that hinder the pantothenic acid utilization and thus block the parasite life cycle[7]. But due to the poor stability of these carboxylic acids in human serum they are not suitable as clinical candidates.[8–10] Recently, the focus has been shifted towards the synthesis of pantothenamide and inverted pantothenamide analogues (Figure 1).[11–13,14] These inverted amide-bond pantothenamides (in red, Figure 1) are one class of such analogues which possess antiplasmodial activity.[10,12,15–17]

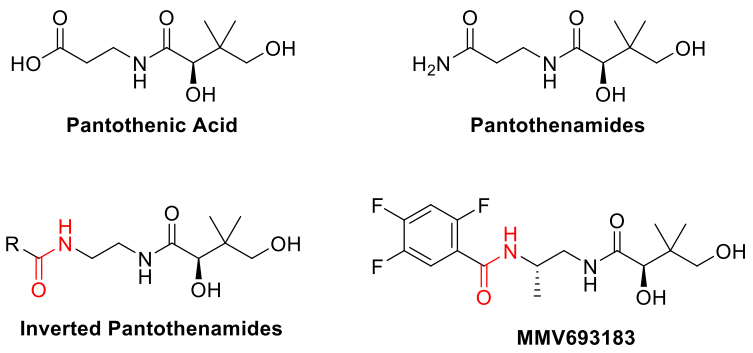
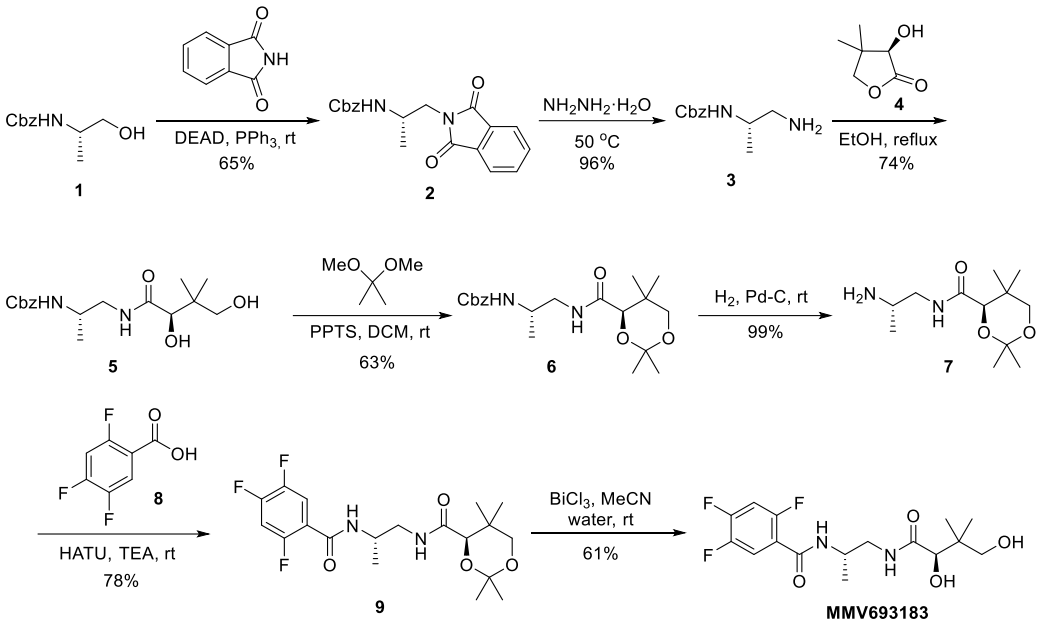


Figure 1. Chemical Structures of MMV693183 and Related Pantothenic Acid Derivatives

Medicines for Malaria Venture (MMV) has developed an analogue of inverted pantothenamides called MMV693183 as a single dose treatment for uncomplicated malaria and for resistance management. MMV693183 has shown a novel mode of action by inhibition of acetyl-CoA synthetase.[13-14] Thus, developing a cost-effective process for the synthesis of MMV693183 will make the therapy more affordable and increase its likely impact. Unfortunately, only one synthetic route for MMV693183 was published so far, and it would be quite limiting to employ it as a production route with a low price point in mind (Scheme 1).[14] This route started with a Mitsunobu reaction of Cbz-protected aminoalcohol **1** with phthalimide to provide **2** in 65 % yield, which was immediately subjected to phthalimide deprotection to provide the mono-protected diamine **3** in 96%. The resulting Cbz-protected diamine **3** was then reacted with (*R*)-pantolactone (**4**) to afford the diol **5** in 74 % yield, which was then protected by 2,2-dimethoxypropane to provide acetonide **6** in 63 % yield. Cbz-deprotection by hydrogenolysis provided the free amine **7** in quantitative yield, which allowed for selective acylation of the amine with 2,4,5-trifluorobenzoic acid (**8**) to provide amide **9** in 78 % yield. Finally, the acetonide protecting group was removed to furnish MMV693183 in 61 % yield (14 % overall yield).



Scheme 1. Reported synthetic route to MMV693183

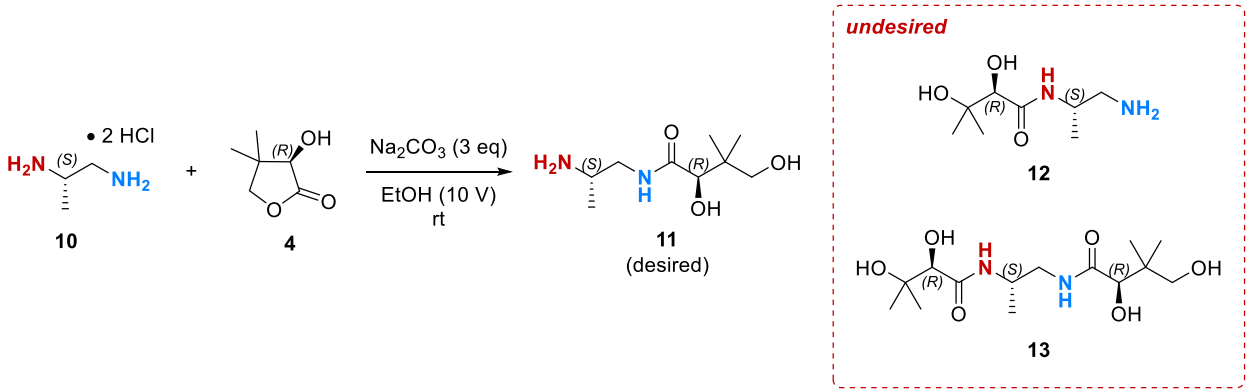
While this 7-step sequence was successfully employed to make the MMV693183 on decagram scale it also offers several opportunities for improvement. For example, the API was only provided in an overall yield of ~14 % and 4 of the 7 steps were used to manipulate protecting groups. The introduction of the less sterically hindered primary amine was accomplished via the Mitsunobu reaction and subsequent hydrazine deprotection which are challenging transformations to scale-up due to their inherent wastefulness, cost, and the safety risks associated with handling diazodicarboxylates and hydrazine at scale. Thus, a more efficient and scalable route is needed for synthesis of MMV693183 that would accommodate cost-effective commercial implementation and maximize access to this drug should it become commercially available.

RESULTS AND DISCUSSION

Herein, we report a 3-step scalable synthesis of MMV693183 using readily available and low-cost starting materials, which avoids the use of any protecting group. Our approach is based

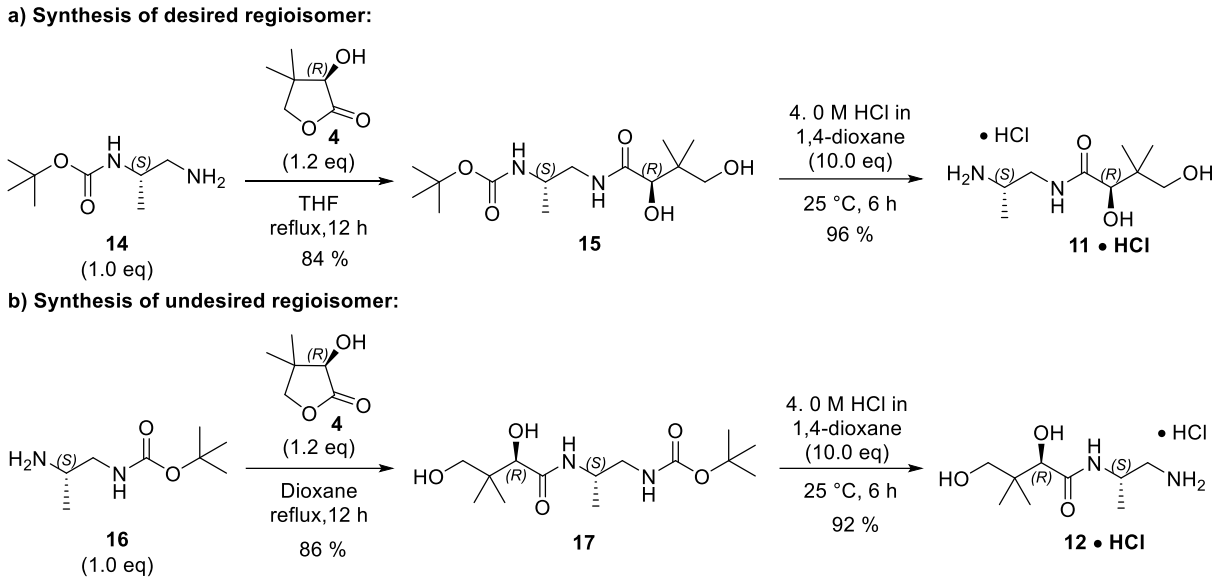
on the hypothesis that the steric differences of both the primary amines in (*S*)-1,2-diaminopropane dihydrochloride (**10**) would alone be sufficient to direct acylation to the desired less hindered amine (in blue) in a regioselective fashion (Table 1). To test this hypothesis, amine **10** (readily available by resolution of the corresponding racemic amine)[18–20] was reacted directly with (*R*)-pantolactone (**4**), in the presence of 3 eq of Na_2CO_3 to liberate the free amine of **10** (Table 1, Entry 1). In this initial reaction we observed good reactivity of the starting diamine, however, the mixture

Table 1. Direct Amidation of Diamine with (R)-Pantalactone[a]



Entry	Solvent	Concentration (V)	Base	T (h)	HPLC (A %)[b]		HILIC (A % ratio)[c]
					13	11 + 12	11 : 12
1	EtOH	10 V	Na_2CO_3	3	8	52	89 : 11
2	MeOH	10 V	Na_2CO_3	3	4	82	90 : 10
3	IPA	10 V	Na_2CO_3	72	8	41	90 : 10
4	THF	10 V	Na_2CO_3	3	--[e]	--	--
5	CH_3CN	10 V	Na_2CO_3	3	--[e]	--	--
6	DMF	10 V	Na_2CO_3	3	--[e]	--	--
7	DMSO	10 V	Na_2CO_3	3	--[e]	--	--
8	IPA : H_2O (7 : 3)[d]	10 V	Na_2CO_3	3	5	85	91 : 09
9	IPA : H_2O (7 : 3)	5 V	Na_2CO_3	3	7	84	90 : 10
10	IPA : H_2O (7 : 3)	20 V	Na_2CO_3	3	6	84	92 : 08
11	IPA : H_2O (7 : 3)	10 V	Et_3N	3	6	88	91 : 09
12	IPA : H_2O (7 : 3)	10 V	$NaHCO_3$	3	--[e]	--	--
13	IPA : H_2O (7 : 3)	10 V	$NaOCH_3$	6	45	55	90 : 10
14	**IPA : H_2O (9 : 1)**	**10 V**	**Na_2CO_3**	**3**	**4**	**86**	**90 : 10**
15[f]	IPA : H_2O (9 : 1)	10 V	Na_2CO_3	6	11	86	90 : 10

[a]All reactions were carried out with (*S*)-propane-1,2-diamine dihydrochloride **10** (1.0 g 1.0 eq), (*R*)-3-hydroxy-4,4-dimethyldihydrofuran-2(3H)-one **4** (1.0 eq.), base (3,0 eq), 25 °C, 10 V of solvent unless otherwise stated. [b]LCAP at 210 nm. [c]HILIC ratio at 210 nm. [d]IPA: iPrOH. [e]No reaction. [f]Reaction was carried out at 0 °C.



Scheme 2. Synthesis of Regioisomers for HPLC Standards

of amide products formed in the reaction was difficult to quantify and characterize. In order to deconvolute the reaction mixture, we next discretely synthesized compounds **11** and **12** to utilize as analytical standards (Scheme 2). These were prepared by reaction of (*R*)-pantolactone (**4**) with both the Boc-protected amines **14** and **16** followed by Boc deprotection to provide pure **11** and **12** as their HCl salts.

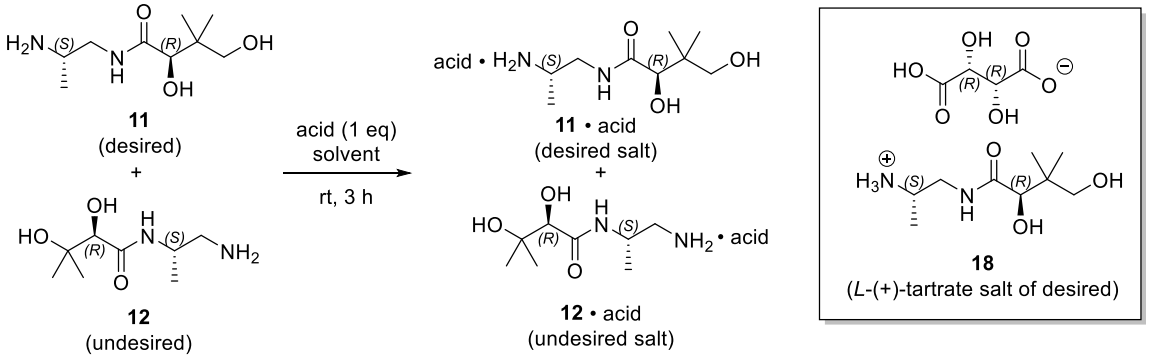
Having the standards in hand allowed for the development of an HPLC method for identification of the ratio of products in each direct amidation reaction (Table 1). A standard reverse phase HPLC method was developed that was capable of separating the diamide products **13** from **11** and **12**, but could not competently separate the regioisomers **11** from **12**. A Hydrophilic Interaction Liquid Chromatography (HILIC) method, however, was able to separate both regioisomers (**11** and **12**) and thus both methods were used to characterize the reaction mixtures formed. The initial reaction (Table 1, Entry 1) with 3.0 eq of Na_2CO_3 provided 52 % assay yield

(by HPLC A%) of a 9:1 regioisomeric mixture of monoamide products (**11** and **12**) favoring the desired product. It was also observed, that 8 % of the diacylated side product **13** (separated in the standard HPLC method) formed in this reaction. For further optimization, a systematic solvent screening was conducted utilizing Na_2CO_3 as the base (Table 1, Entries 2−8). It was determined that the reaction occurred only in polar protic solvent, such as MeOH, EtOH and *i*-PrOH (Table 1, Entries 1-3), but no reaction was observed in polar aprotic solvent probably due to insolubility of the inorganic base (Table 1, Entries 4-7). In *i*-PrOH the reaction was significantly slower, however, the addition of water to the solvent system (Entries 9-15) gave superior results and up to 86 % (HPLC A%) of the monoamide products (Table 1, Entry 8). Notably, the regioisomeric ratio was consistently 9:1 in favor of the desired regardless of the conditions screened. A standard 10 V concentration of solvent was necessary to ensure proper mixing of the reaction mass as the reaction mixture in 5 V conditions (Table 1, Entry 9) was difficult to stir. More diluted conditions (20 V of solvent) gave the same results as 10 V of the solvent (Table 1, Entry 10) and thus 10 V was deemed ideal. With this optimized solvent system in hand, various bases were screened (Table 1, Entries 11-13). Among these different bases, Na_2CO_3 and Et_3N offered the best results, however, Et_3N proved difficult in the workup presumably due to the formation of the Et_3N hydrochloride salt. Notably, the mixed solvents of *i*-PrOH:H_2O (7:3) worked well, however, product isolated from this mixed solvent system was always of relatively low purity. The product was contaminated with inorganic salts, likely due to the high-water content in the reaction mixture leading to isolation of the water-soluble product mixture along with significant amounts of dissolved inorganic salts. Decreasing the amount of water in the reaction from 7:3 to 9:1 (*i*-PrOH:H_2O) allowed for effective isolation of the product with a high level of purity (**11** + **12** > 95 %, qNMR) (Table 1, Entry 14). Ultimately, the optimal conditions for this reaction (Table 1, Entry 14) provided the mixture (~9:1)

of monoamide products **11** and **12** in 86 % yield after column chromatography. The mixture of monoamides (**11** and **12**) was then taken on to the next step for further purification.

With conditions to provide predominately the desired amide product **11**, the purification of the mixture to exclude the undesired secondary amide **12** (Table 2) was studied. The free amines are liquids after chromatography, but for depletion of the undesired isomer **12** a crystallization was sought. Thus, the 9:1 mixture of **11** and **12** was treated with various acids to screen for a suitable recrystallization method. To this end, several mineral acids were initially tried to form the corresponding amine salts, however, in all cases the freshly formed amide bond was cleaved under these conditions (Table 2, Entries 1-3).

Table 2. Purification of 11:12 Monoamide Mixture[a]



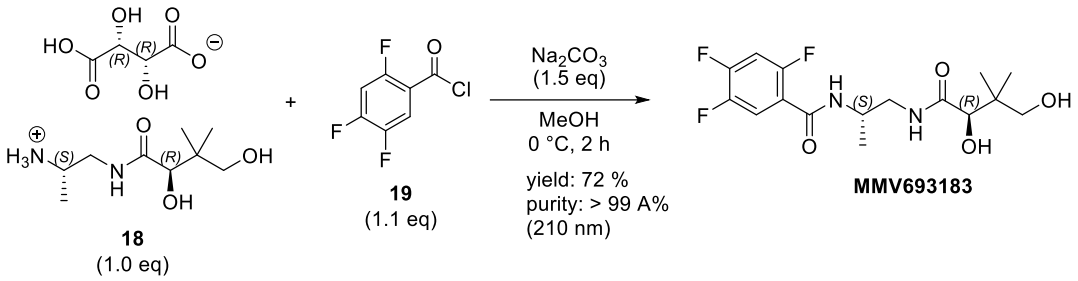
Entry	Acid	Solvent	Results	HILIC (A % ratio)[b]
				11 : 12
1	4.0 M HCl	1,4-dioxane	Amide cleaved	ND
2	1.2 M HCl	IPA	Amide cleaved	ND
3	H_2SO_4	EtOH	Amide cleaved	ND
4	H_3PO_4	EtOH	No Precipitate	NA
5	Formic Acid	EtOH	No Precipitate	NA
6	Benzoic Acid	EtOH	No Precipitate	NA
7	Citric Acid	EtOH	No Precipitate	NA
8	*D*-(-)-Tartaric Acid	EtOH	No Precipitate	NA
9[c]	***L*-(+)-Tartaric Acid**	EtOH	**Stable White Salt**	**25:1**
10	*L*-(+)-Tartaric Acid	Methanol	No Precipitate	NA
11	*L*-(+)-Tartaric Acid	Acetone	No Precipitate	NA
12	*L*-(+)-Tartaric Acid	EtOAc	No Precipitate	NA

13[d]	*L*-(+)-Tartaric Acid	iPrOH	Hygroscopic White Salt	98:2
14[d]	*L*-(+)-Tartaric Acid	**iPrOH:MeOH (9:1)**	**Stable White Salt**	**100:0**

[a]All the reactions were carried out with ~9:1 regioisomeric mixture of **11**:**12** (1.0 g, 1.0 eq), acid (1.0 eq), Solvent (10 V), rt, 3h. [b]HILIC ratio at 210 nm. ND = Not Determined. NA = Not Applicable. [c]80 % of mass recovery. [d]78 % of isolated yield.

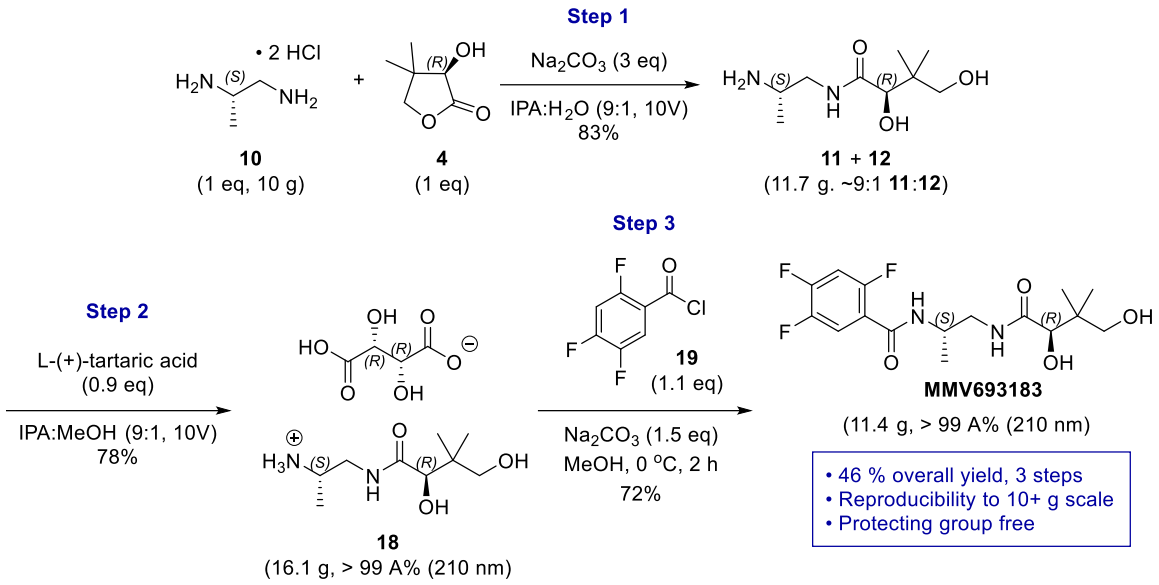
Under less acidic conditions using organic acids the amide bond proved stable, however, most resulting salts that formed were not crystalline making them unsuitable for further purification (Table 2, Entry 4-8). Intriguingly, when *L-(+)*-tartaric acid was utilized, a stable white solid (**18**) was formed and more importantly, the desired regioisomer was enriched to greater than 25:1 after recrystallization providing ~80 % mass recovery (Table 2, Entry 9). Different solvents to further enhance the mass recovery of **18** were screened. Among a variety of solvents screened for salt formation, it was found that co-solvents of *i*-PrOH:MeOH (9:1) gave a stable tartrate salt of the desired regioisomer (Table 2, Entry 14), while other solvent systems where less effective. The formation of the *L-(+)*-tartrate salt (**18**) rejected the undesired regioisomer effectively affording exclusively the desired regioisomer along with some excess *L-(+)*-tartaric acid in the isolate. Additional *L-(+)*-tartaric acid created purification problems in the following step, thus, the amount of *L-(+)*-tartaric acid used was optimized. Ideal conditions for the desired amine salt **18** were achieved using 0.9 eq of the *L-(+)*-tartaric acid providing **18** in 78 % yield as the exclusive regioisomer.

To complete the synthesis of MMV693183, *L-(+)*-tartrate salt **18** was reacted with 2,4,5-trifluorobenzoyl chloride (**19**) in the presence of 1.5 eq Na_2CO_3 providing the final API in 72 % yield in > 99 A% (210 nm) purity as determined by HPLC (Scheme 3). Both potassium and sodium carbonate can be used as a base to promote this final reaction and both provide similar results, however Na_2CO_3 is lower cost and was ultimately preferred.



Scheme 3. Completion of MMV693183 Synthesis

To further showcase the synthetic utility of our three-step protocol for preparation of MMV693183, two 10+ gram scale batches were carried out (Scheme 4). Starting with 10 g of the (*S*)- 1,2-diaminopropane dihydrochloride (**10**), the monoamide product was isolated as a regioisomeric mixture (~9:1) in an 83 % yield after a column purification to remove the diamide impurity. Future work will explore the possibility to omit a column chromatography. Treatment of the mixture of **11** and **12** with 0.9 eq of *L-(+)*-tartaric acid furnished the pure *L-(+)*-tartrate salt **18** in 78 % yield with > 99 A% (210 nm) HPLC purity. The resulting salt **18** was then acylated with 2,4,5-trifluorobenzoyl chloride (**19**) to afford MMV693183 in 72 % yield with > 99 A% (210 nm) HPLC purity. The overall yield of the three-step process from **10** to make MMV693183 was 46 %.



Scheme 4. Gram-Scale Demonstration of Protecting Group-Free Synthesis of MMV693183

CONCLUSION

Contrasting this newly developed synthetic route of MMV693183 with the prior reported route, a number of critical advantages have been achieved in this body of work, including: 1) dramatically shortening the number of steps required to make MMV693183 with a considerably higher overall yield from 7 to 3; 2) eliminating the need for wasteful and expensive usage of protecting groups by taking advantage of the native steric differences of the primary amines in **10** and removing the need for an acetonide which was ultimately unnecessary given the disproportionally greater reactivity of the primary amine; 3) the discovery of *L-(+)*-tartaric acid to allow for depletion of the undesired regioisomer, and 4) utilizing 2,4,5-trifluorobenzoyl chloride for ultimate acylation to avoid using expensive coupling reagents, all of this resulting in a cost-effective and scalable strategy to this promising API. These findings will hopefully serve to improve the commercial-scale manufacturing of MMV693183 in its effort to combat malaria.

SUPPORTING INFORMATION

The Supporting Information is available free of charge at https://pubs.acs.org/doi/SOMEJOURNALLINK These data include additional experimental details, analytical methods.

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