Studies Towards the Total Synthesis of MPC1001F

by

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Abstract

This thesis describes the studies towards the total synthesis of MPC1001F, a triketomonothiopiperazine natural product. In section **2.1**, the first route, based on previous studies, reached the pyrrolidinone/diketopiperazine system with correct stereochemistry but did not go to completion due to the poor transformation of diketopiperazine ring synthesis by Dieckmann cyclization. Then, in section **2.2**, a series of studies on the modification of the Dieckmann cyclization were conducted and finally, in section **2.3**, a new route to the pyrrolidinone/diketopiperazine ring system of MPC1001F was developed and the precursor of the triketomonothiopiperazine skeleton was synthesized. This route also found the solutions of the potential problems on the MPC1001F synthesis, like electrophilic sulfenylation, diketopiperazine ring formation and hydroxyl group installation was correct stereochemistry.

Acknowledgement

I would like to thank Prof. Derrick L. J. Clive for the supervision in the past three years. I learnt a lot of chemistry under his guidance and became stronger in the synthesis research.

I also appreciate the analytic support from the chemistry department and at last I would like to thank the assistance from the group members and the support from my family.

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List of Abbreviations

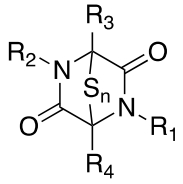
Ac	acetyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
t-Bu	*tert*-butyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DKP	diketopiperazine
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin Periodinane
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide
ETP	epipolythiodioxopiperazine
HOBt	hydroxybenztriazole
KHMDS	potassium hexamethyldisilazide
LDA	lithium diisopropylamide
LiHDMS	lithium hexamethyldisilazide
nM	nanomolar
pyr	pyridine
Pg	protecting group
ppm	parts per million

TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl
TFAA	trifluoroacetic anhydride
TLC	thin layer chromatography
TFA	trifluoroacetic acid
TMS	trimethylsilyl
TsOH	*p*-toluenesulfonic acid

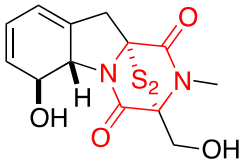
1. Introduction

1.1 Introduction to the MPC1001 family of natural products

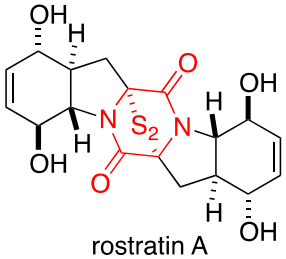
Epipolythiodioxopiperazine (ETP) natural products **1.1** (Scheme 1) are a very important class of fungal metabolites with remarkable bioactivities against viruses, bacteria and fungi, etc.[1] Additionally, the ETP core, which is fused to other different size rings and functional groups, occurs in a large number of natural products with diverse complex structures (**1.2**–**1.5**). Because of the important bioactivities and beautiful structures, these molecules have attracted many organic chemists to conquer their total synthesis in recent decades.[2]



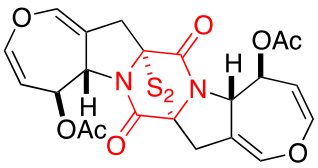
ETP core
1.1



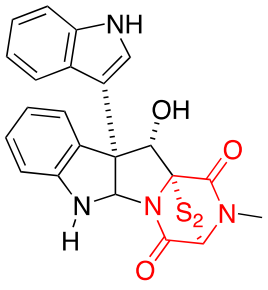
gliotoxin
1.2



rostratin A
1.3



(-)-acetylaranotin
1.4



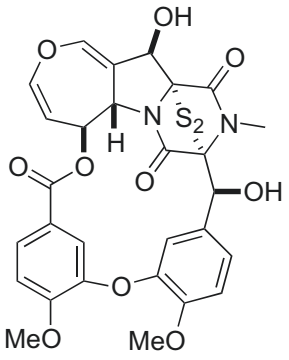
(+)-bionectin A
1.5

Scheme 1.

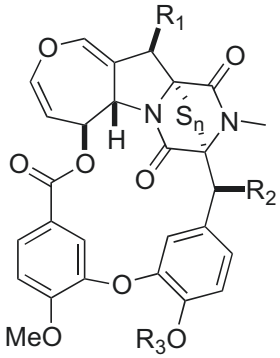
MPC1001 **2.1**, a new member of the ETP natural product family, was first isolated from a microorganism, *Cladorrhinum* sp. KY4992, in 2004 by a Japanese company during their screening for antitumor antibiotics.[3] Subsequently, MPC1001 and its analogues MPC1001B–H (**2.2**–**2.5**) were isolated by the same company from the fermentation mycelium using chromatography techniques, and their structures and stereochemistry were characterized through various NMR experiments.[4]

Structurally, MPC1001 **2.1** has an epidithiodioxopiperazine core fused to a pyrrolidine and a 4,5-dihydooxepin ring, and also has a macrocyclic skeleton. MPC1001B, C, D belong to the same type of structure as MPC1001 (Type I) and show slight differences in the R_1, R_2 and R_3 groups or in the number of sulfurs in the bridge over the diketopiperazine skeleton. Type II MPC1001 analogues do not contain the polythio bridge and the R_1 group but a double bond instead (**2.3**). The macrocyclic skeleton is not present in type II analogues and the aromatic side chain is terminated with an aldehyde. Moreover, the R_4 group varies from ketone (MPC1001H) to thione (MPC1001G). For type III MPC1001 analogues (**2.4**), the macrocyclic is absent and a tetrathio bridge spans the dioxopiperazine. MPC1001F (**2.5**), the last type of MPC1001 analogue, has a different monothiotrioxopiperazine motif.

Type I:



MPC1001 **2.1**



MPC1001B/C/D **2.2**

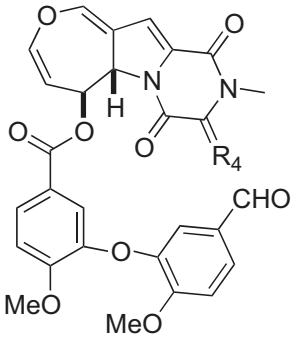
MPC1001B: n = 2; R_1: H; R_2: OH; R_3: Me;

MPC1001C: n = 2; R_1: OH; R_2: H; R_3: Me;

MPC1001D: n = 3; R_1: OH; R_2: OH; R_3: Me;

Emestrin: n = 2; R_1: OH; R_2: OH; R_3: H

Type II:

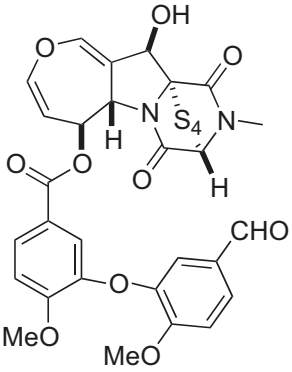


MPC1001G: R_4: S

MPC1001H: R_4: O

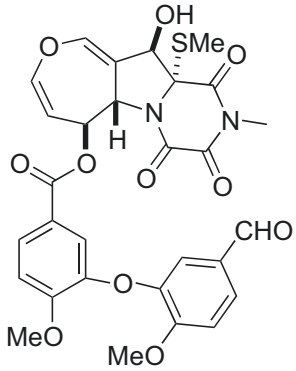
MPC1001G/H **2.3**

Type III:



MPC1001E **2.4**

Type IV:



MPC1001F **2.5**

Scheme 2.

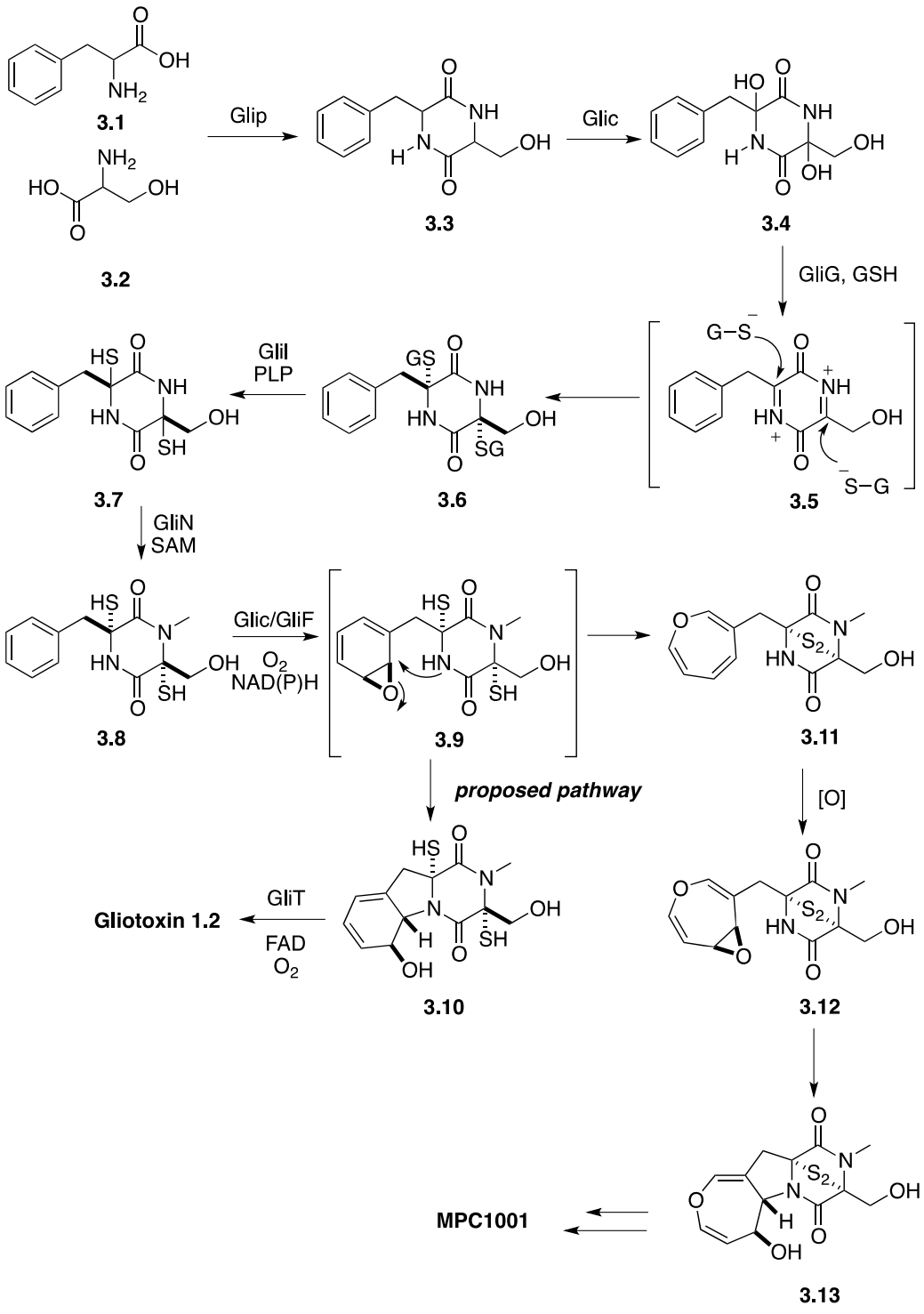
1.2 Biological aspect of the MPC1001 family of natural products

In terms of bioactivities, MPC1001 and its analogues, except for MPC1001F, exhibit different levels of antiproliferative activity against the human prostate cancer cell line (DU145) (Table 1).[4] Specifically, MPC1001 has a very good IC_{50} value of 9.3 nM and other type I analogues also show satisfactory results (MPC1001B 39.0 nM; MPC1001C 12.0 nM; MPC1001D 16.0 nM). Type III analogue MPC1001E shows a decrease in IC_{50} to 83 nM and the type II analogues MPC1001G and MPC1001H give even lower IC_{50} values from 350.0 nM to 450.0 nM. Combining the activities with the structures of MPC1001 molecules and the reported literature,[5] we suspect that the disulfide bridge and the macrocyclic skeleton make the most contributions to the bioactivities.

Table 1.

Compounds	IC_{50} (nmol/L)
MPC1001	9.3
MPC1001B	39
MPC1001C	12
MPC1001D	16
MPC1001E	83
MPC1001G	350
MPC1001H	450
MPC1001F	—

The biosynthesis of MPC1001 has not been reported but some clues can be obtained from the biosynthesis pathway of other ETP natural products. The most widely studied ETP natural product is gliotoxin and its biosynthesis pathway is summarized below (Scheme 3).[6] The diketopiperazine skeleton **3.3** is synthesized from phenylalanine **3.1** and serine **3.2**[6a] and then the enzyme Glic is involved in the dihydroxylation of the DKP ring[6b] to give the intermediate **3.4**. This active intermediate loses water and forms the imine **3.5**, and thiolation (GliG-mediated using GSH) then yields the disulfide compound **3.6**. Then the Glil catalyzes **3.6** to give dithiol intermediate **3.7**. Selective *N*-methylation catalyzed by GliN affords **3.8** and Oxidation of the phenyl ring, proposed by phenyl oxide formation and cyclization via nucleophilic attack, affords reduced gliotoxin **3.10**,[6c] which can be transformed to gliotoxin **1.2** by GliT oxidation.[6d]



Scheme 3.

GliP = gliotoxin peptide synthetase; GliC = gliotoxin cytochrome P450 enzyme; GliG = a gene enabled to code for a glutathione-S-transferase; GliM = gliotoxin

methyl transferase; GliT = gliotoxin thioredoxin reductase; GSH = glutathione.

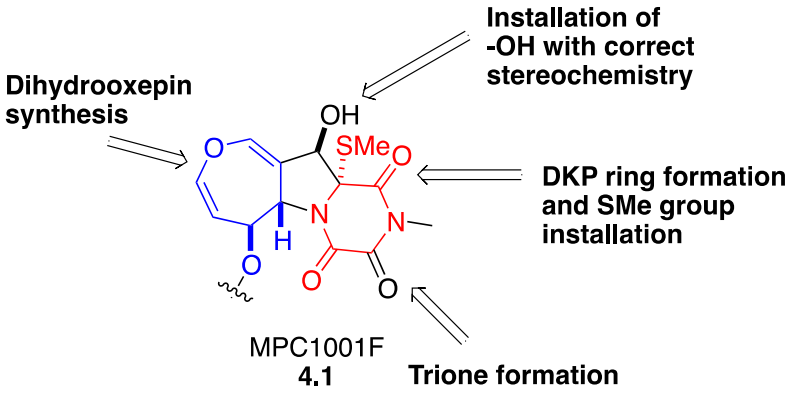
The biosynthesis of MPC1001 could be hypothesized to occur in a similar way. For the dihydrooxepin ring formation, a proposed route involving a [3,3] sigmatropic rearrangement (**3.9**→**3.11**) and a second epoxidation (**3.12**), followed the nucleophilic attack, would give the dihydrooxepin motif **3.13**[6c]. Rastetter and his coworkers synthesized oxepin oxide and use various nucleophiles to open the epoxide ring.[7] The intramolecular experiment they performed did not afford the desired dihydrooxepin subunit and so the proposed biosynthesis is controversial.

1.3 Current synthetic studies on the MPC1001 and other structurally related molecules

Up to now, no total synthesis of any of the MPC1001 family members has been reported. However, several model studies towards MPC1001, especially those from this laboratory, and total synthesis of other structurally related natural products should provide us with valuable information.

The target of our project is MPC1001F, and there are several challenges to be overcome in our studies on this molecule (Scheme 4): i, dihydrooxepin skeleton synthesis; ii, diketopiperazine construction; iii, installation of the hydroxy group on C-3 with correct stereochemistry; iv, trione formation on the

DKP ring. Herein, the current studies on these potentially challenging steps are briefly summarized.

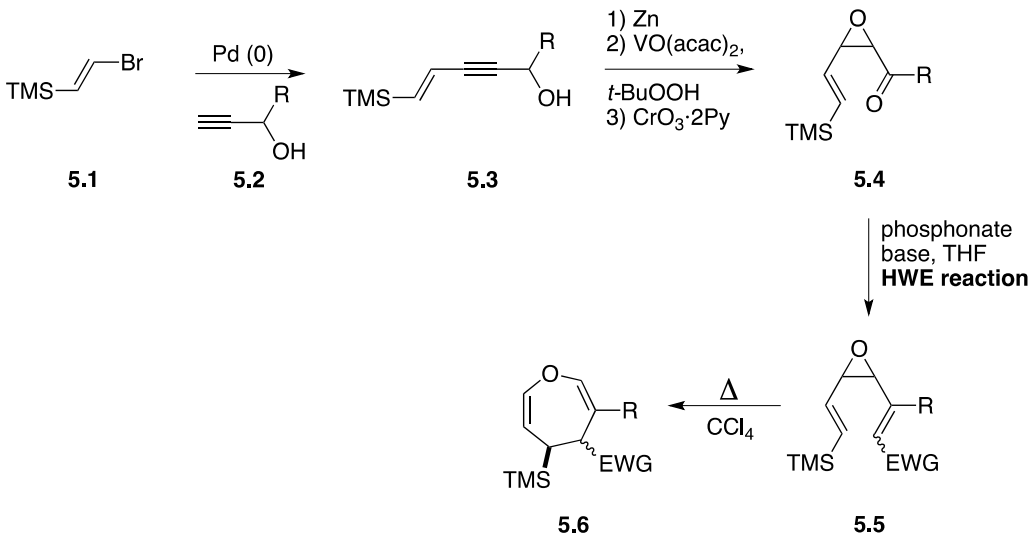


Scheme 4.

1.3.1 Reported studies on the dihydrooxepin synthesis

In the past several decades, several methods have been developed to synthesize the dihydrooxepin skeleton. An early reported method to the dihydrooxepin skeleton was developed by White *et al*[8] through Cope rearrangement of 2,3-divinyl epoxides.

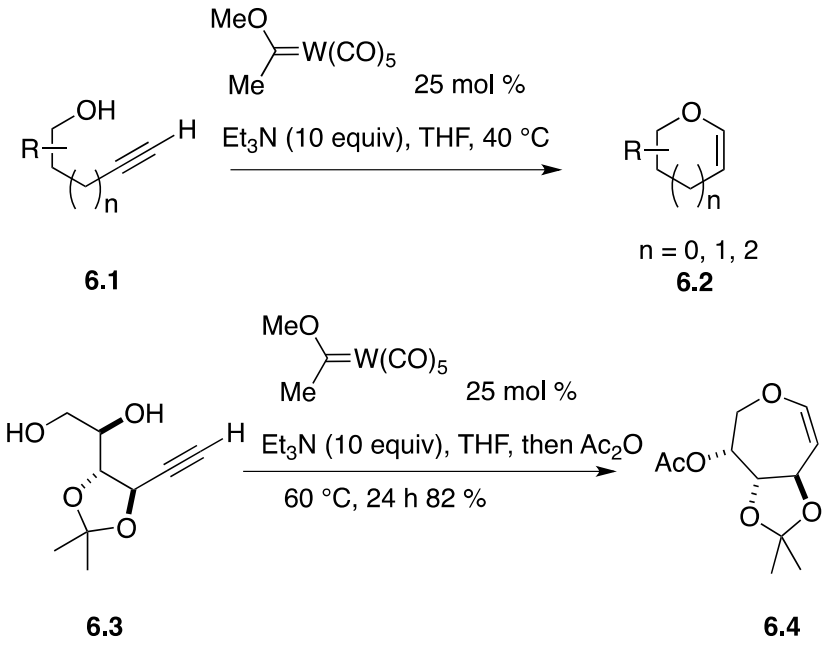
The Sonogashira coupling of vinyl bromide **5.1** with propargylic alcohol **5.2** afforded **5.3**. Then Rieke zinc *cis* reduction of **5.3** gave the cis diene. This step was followed by hydroxyl group directed epoxidation and oxidation, which gave **5.4**. Horner-Wadsworth-Emmons olefination created the extended C=C bond in **5.5** which was ready to undergo a [3,3] sigmatropic rearrangement under thermal conditions to yield the dihydrooxepin ring system **5.6**.



5.1 **5.2** **5.3** **5.4** **5.5** **5.6**

Scheme 5.

In 2007, McDonald's group developed a tungsten-catalyzed cycloisomerization of alkynyl alcohols[9] via Fischer carbene formation, which could be used to construct endocyclic enol ethers of different sizes from five to seven membered (Scheme 6).



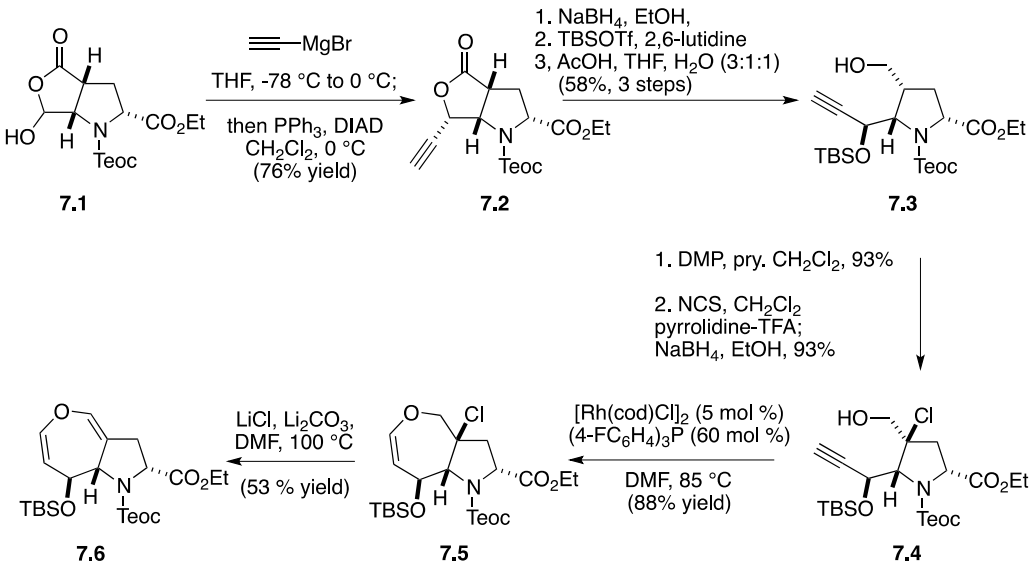
Scheme 6.

Specifically, under these conditions, diol **6.3** was transformed into the seven-membered enol ether **6.4**, whose formation was more favorable than a five-membered enol ether. This seven-membered enol ether could, in principle, be a good precursor to the dihydrooxepin ring.

Similarly, in 2012, Reisman's group reported the first total synthesis of (−)-acetylaranotin, a dihydrooxepin epidithiodiketopiperazine natural product.[2b] The key pyrrolidine intermediate **7.4** underwent a Rh(II) catalyzed cycloisomerization, and this was followed by chloride elimination to afford the dihydrooxepin skeleton **7.6**.

To achieve these transformations, **7.1** was synthesized in four steps from commercial starting materials. This hydroxylactone was treated with an excess of ethynylmagnesium bromide and then with triphenylphosphine/DIAD to

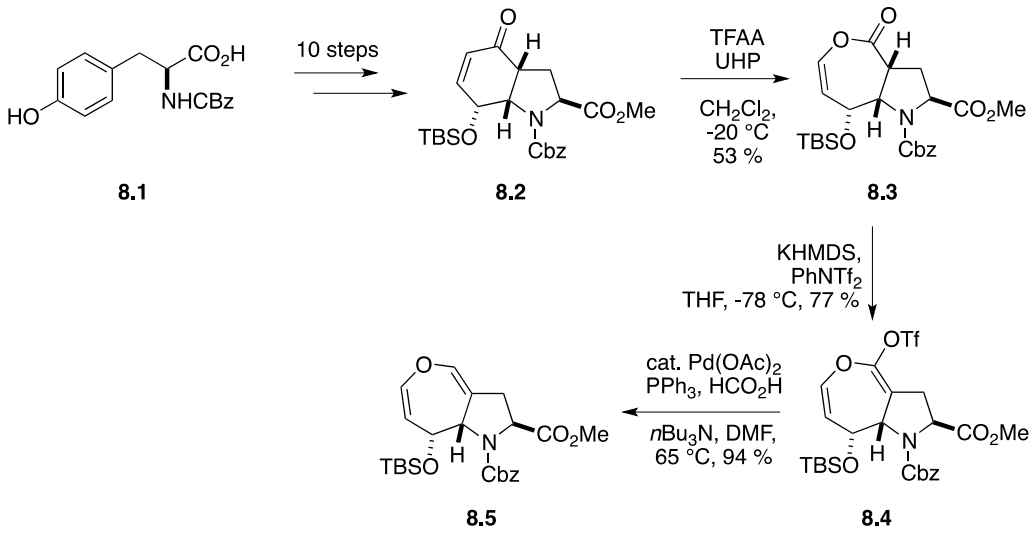
mediate an intramolecular Mitsunobu reaction that delivered the lactone **7.2**. $NaBH_4$ reduction released the free diol and *t*-$BuMe_2SiOSO_2CF_3$ was then used to protect both hydroxyl groups. Then regioselective deprotection gave the primary alcohol **7.3** and left the secondary protected alcohol unaffected. Finally, oxidation of the alcohol to an aldehyde made it possible to install the chlorine on C-3, and reduction of the aldehyde afterwards gave **7.4**.



Scheme 7.

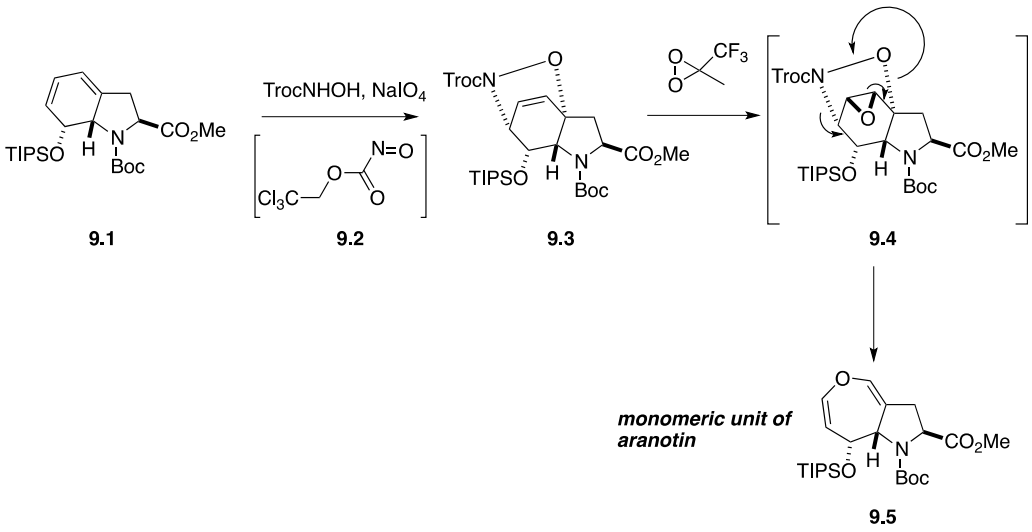
Another approach to make the dihydrooxepin skeleton is from an enol lactone. In 2012, Tokuyama's group completed the total synthesis (-)-acetylaranotin and the dihydrooxepin skeleton was made through this method.[10] Baeyer–Villiger oxidation of the functionalized cyclohexenone **8.2**, using $(CF_3CO)_2O$ and urea hydrogen peroxide (UHP), gave the enol lactone **8.3**. Then

enol formation with strong base (e.g. KHMDS) followed by transition metal catalyzed reduction afforded the dihydrooxepin ring **8.5**.



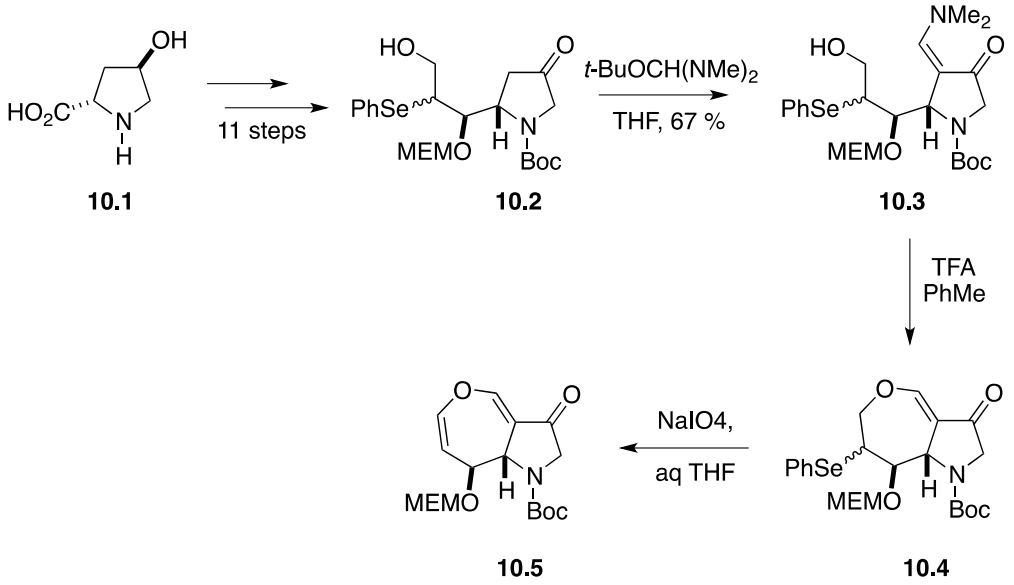
Scheme 8.

Nicolaou's group also invented an approach based on ring expansion, in this case, of a nitroso epoxide to build the dihydrooxepin motif.[11] Trichloroethyl nitrosoformate **9.2**, which was generated in situ from TrocNHOH and $NaIO_4$, was reacted with the cyclic diene **9.1** to afford Diels-Alder product **9.3** with good regio- and diastereoselectivity. Epoxidation of **9.3** followed by ring expansion, presumably through a retro-Diels-Alder pathway, gave the dihydrooxepin **9.5** directly.



Scheme 9.

During research on MPC1001 in this group, Dr. J. Peng developed an acid catalyzed cyclization to make the dihydrooxepin skeleton.[12]



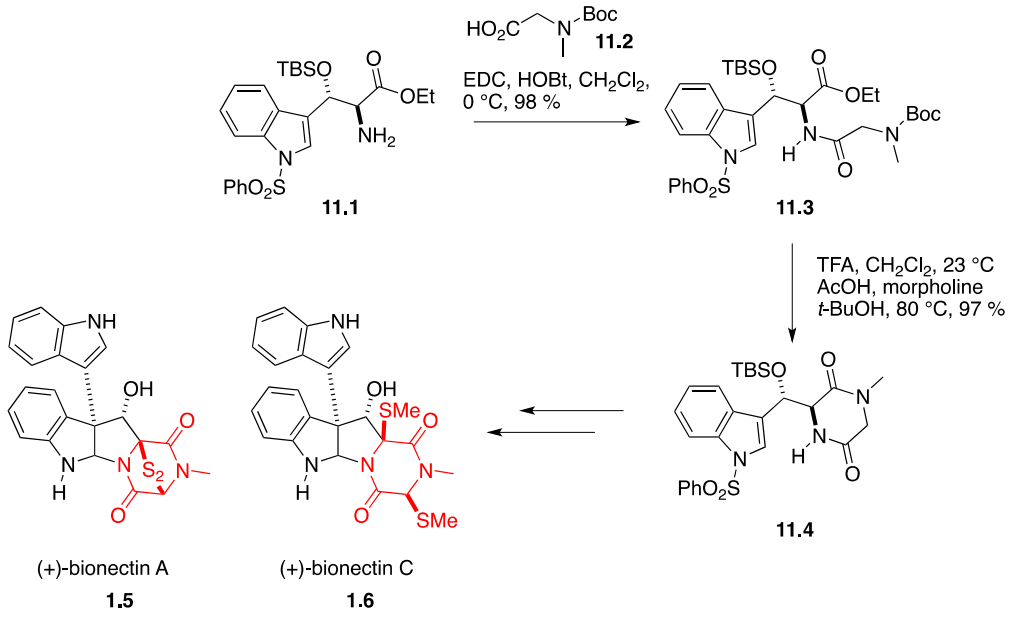
Scheme 10.

Several modifications to the side chain of hydroxyproline **10.1** gave the intermediate **10.2**. Bredereck's reagent was then used to install the dimethylaminomethylene group next to the ketone so as to form the vinylogous amide **10.3**. Acid mediated addition/elimination generated the seven-membered enol ether **10.4**. Finally, oxidative elimination gave the desired dihydrooxepin **10.5**.

1.3.2 Reported studies on the synthesis of diketopiperazines

The diketopiperazine system (DKP) is an important substructure in natural products. The synthesis of the DKP ring has been reviewed by Borthwick[13] and herein the methods used to synthesize the DKP structure during studies on MPC1001 and other similar natural products (e.g. aranotin, bionectin etc.) studies are summarized.

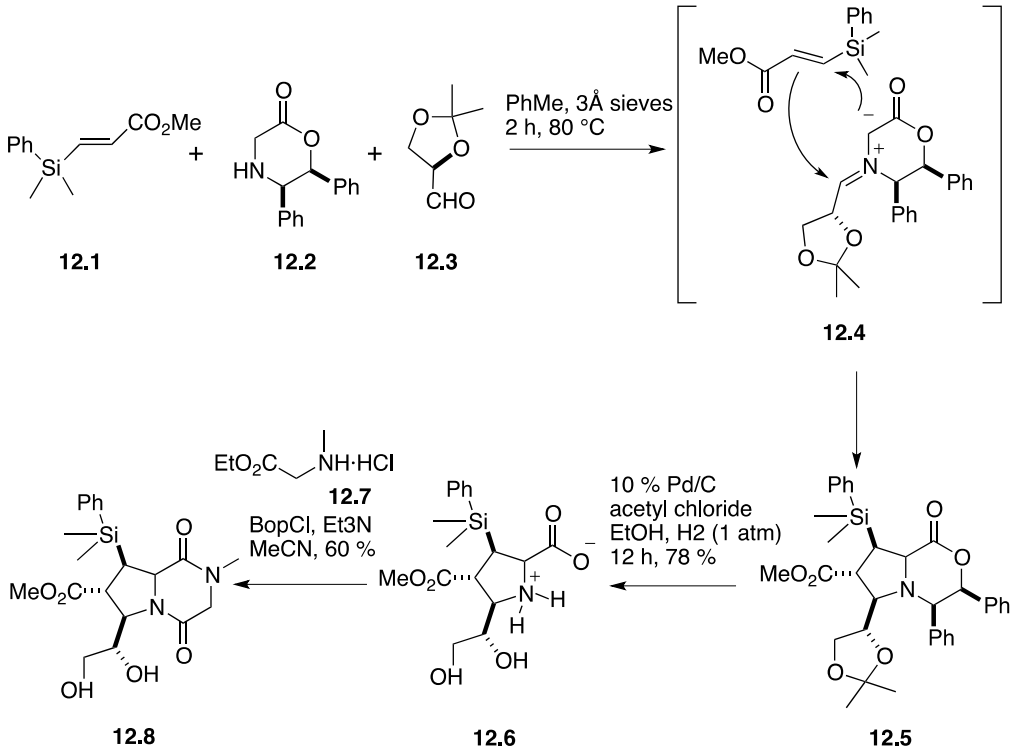
The most common approach to make the DKP ring is the intramolecular amidation or dimerization of the glycine motif. During the synthesis of (+)-bionectins A and C,[2e] the Movassaghi group used **11.1** to couple with *N*-Boc-sarcosine **11.2** and then they conducted a deprotection/condensation to build the DKP skeleton **11.4**.



Scheme 11.

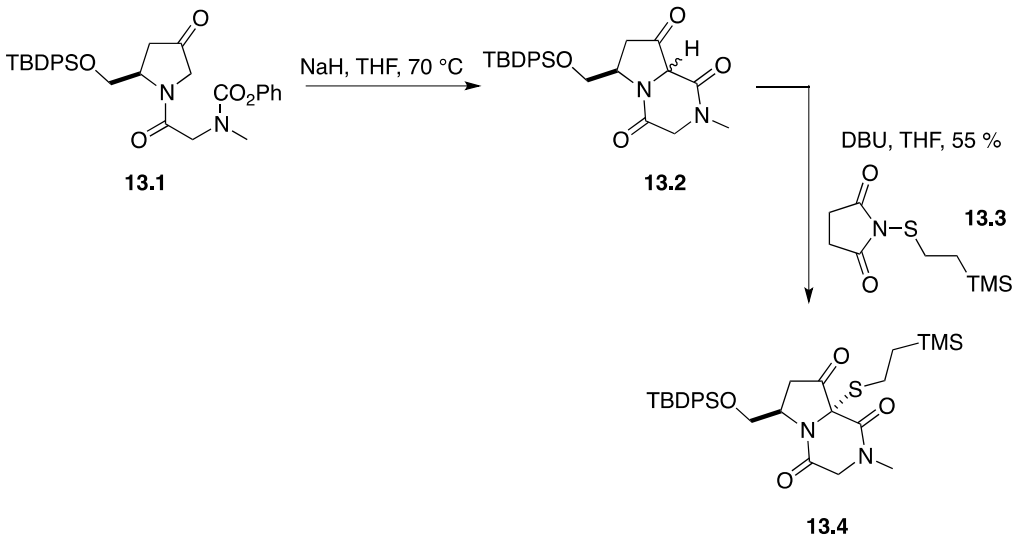
Similarly, this strategy was also used in other DKP-containing natural products like acetylaranotin,[2c] okaramine N[14a] and epicoccin G.[14b]

The Williams's group developed the diphenylmorpholinone auxiliary for the asymmetric synthesis of natural and unnatural amino acids[15] and this chiral auxiliary was successfully utilized to make the BC rings of MPC1001.[16] Firstly, a three-component cycloaddition of **12.1**, **12.2** and **12.3** was carried out and the bicyclic intermediate **12.5** was obtained. Then removal of the auxiliary under reductive conditions and hydrolysis gave the pyrrolidine **12.6**. *N*-Methylglycine ethyl ester **12.7** was then reacted with **12.6** to construct the DKP ring in **12.8**.



Scheme 12.

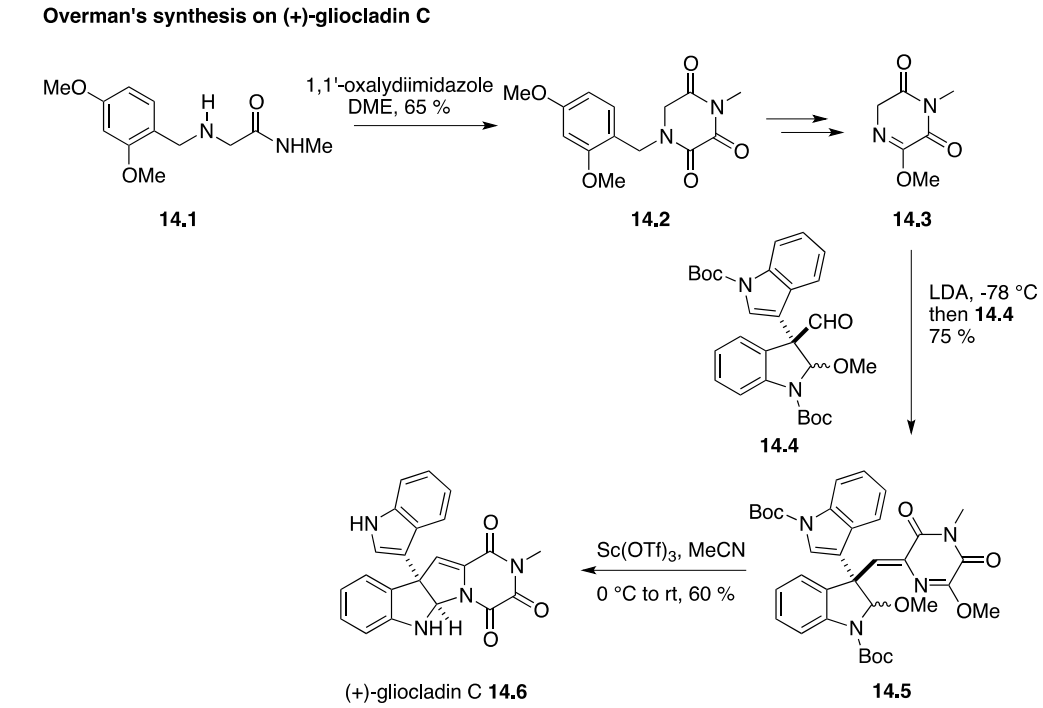
During studies on MPC1001 in this laboratory a Dieckmann type cyclization to make the DKP ring was developed.[12] Ketone **13.1** was treated with NaH in THF to generate the DKP ring intermediate **13.2**. This intermediate was not stable and easily decomposed on silica, but direct treatment with the sulfur electrophile **13.3** under basic conditions led to the stable compound **13.4** with the correct relative stereochemistry.

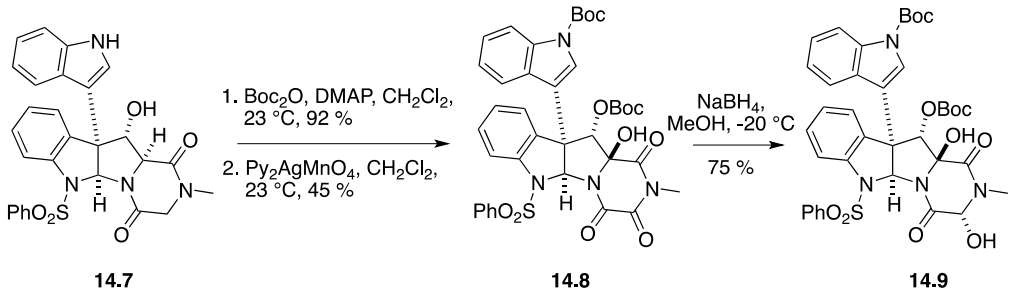


Scheme 13.

Compared with MPC1001, our target MPC1001F has an additional ketone group on the DKP ring. Recent studies shown bellow on this triketopiperazine may give us helpful information for the total synthesis of MPC1001F.

The Overman's group[17] used the compound **14.3** to install the triketopiperazine structure during the total synthesis of (+)-gliocladin C. Compound **14.3** can be synthesized in 3 steps from **14.1**. Then an aldol condensation between **14.3** and aldehyde **14.4** gave **14.5**, which was later smoothly transformed into the triketopiperazine (+)-gliocladin (**14.6**) under Lewis acid catalysis.





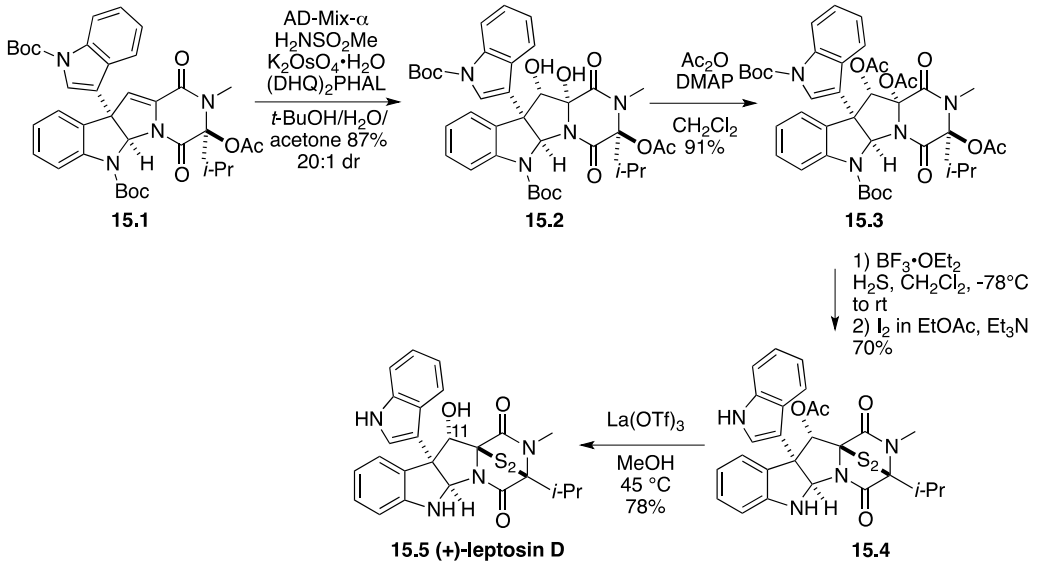
Scheme 14.

In Movassaghi's synthesis on (+)-bionectins A (**1.5**) and C (**1.6**),[2e] triketopiperazine intermediate **14.8** was needed in order to make the α-hydroxy compound **14.9**. Thus, when compound **14.7** was obtained, oxidation using Py_2AgMnO_4 successfully installed the C=O bond on the DKP ring in moderate yield.

1.3.3 Hydroxylation and sulfenylation with trans stereochemistry

It is very challenging to introduce the hydroxyl group on the pyrrolidine ring and also control the stereochemistry with respect to the adjacent sulfur groups (SMe or disulfide bridge). Up to now, only a few papers have focused on this problem.

The Overman group[18] reported the total synthesis of alkaloid (+)-leptosin D (**15.5**) containing the C-11 hydroxyl and its installation was effected through a late-stage diastereoselective dihydroxylation of the pyrroline and the same method was also used to synthesize other two natural products (+)-T988C, (+)-gliocladin A reported in the same paper.

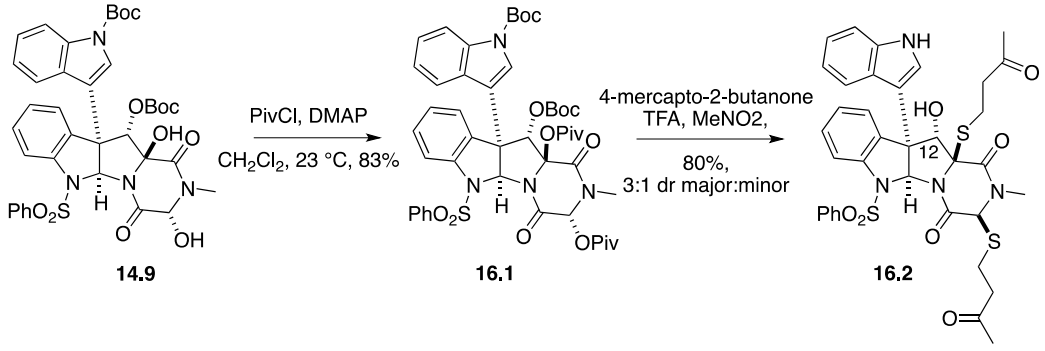


Scheme 15.

Sharpless asymmetric dihydroxylation of **15.1** gave diol **15.2** and then protection by reaction with Ac_2O afforded **15.3**. Lewis acid activation of **15.3**

formed the imine and exposure to the nucleophile H_2S replaced the OAc group with the stereochemical outcome indicated in **15.4**. Oxidation with iodine transformed the intermediate dithiol to the disulfide bridged compound **15.4**, and the final step of deprotection completed the total synthesis of (+)-leptosin D (**15.5**).

In Movassaghi's synthesis of (+)-bionectins A and C[2e], the C-12 hydroxyl group is from commercial starting materials without need for late stage installation (see Scheme 14). Compound **14.9** was treated with PivCl and DMAP to protect the diol (**16.1**). Then trifluoroacetic acid was used for deprotection of **16.1** leading to formation of an imine. 4-Mercapto-2-butanone attacked from the opposite side to the C-12 hydroxyl group, so as to complete the sulfenylation with the right stereochemistry (Scheme 16).

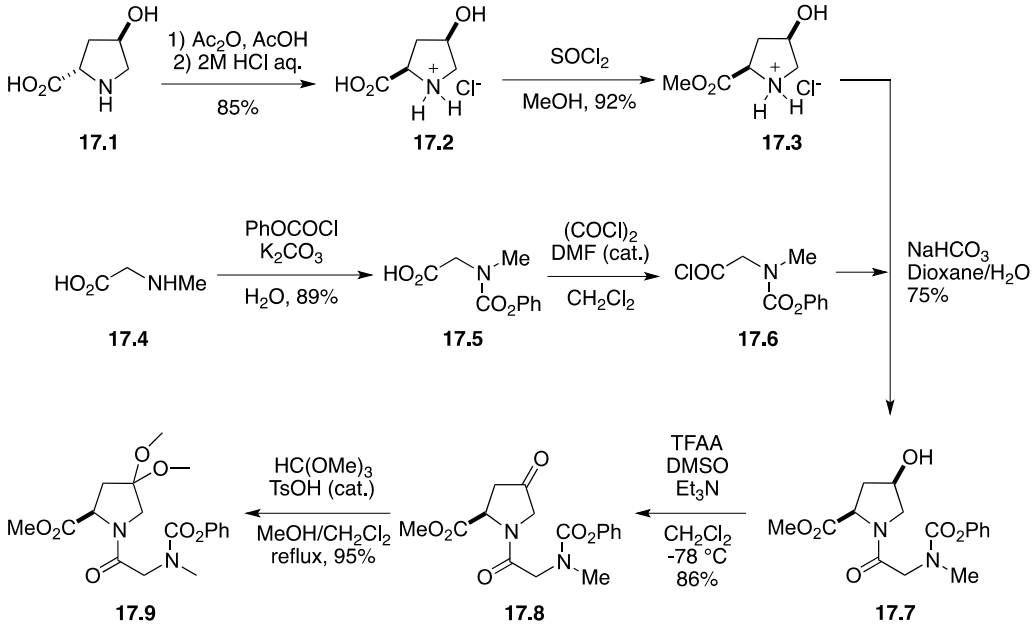


Scheme 16.

2. Results and Discussions

2.1 First attempt on the synthesis of MPC1001F

At the outset, based on previous studies done in this laboratory by Dr. J. Peng[19] and Dr. D. Bhattacharyya,[20] my first approach to MPC1001F started from *trans*-4-hydroxy-L-proline (**17.1**). Epimerization of this compound[21] at C (2) gave the *cis* isomer **17.2**, which was purified by recrystallization to give only a single diastereomer. This stereocenter inversion was done so as to control the stereoselectivity when we install the SMe group at a later stage.



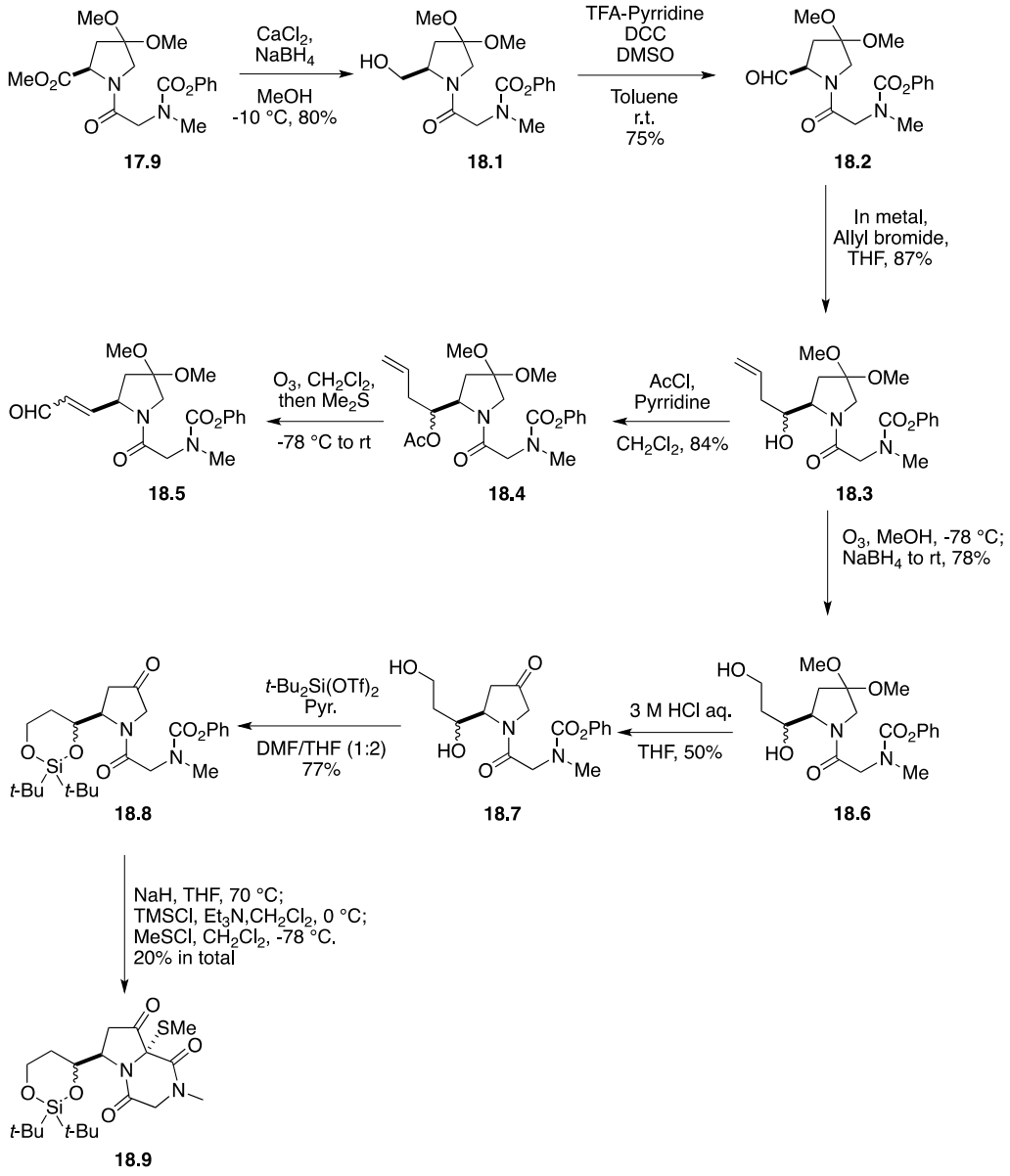
Scheme 17.

Esterification of **17.2** with methanol in the presence of $SOCl_2$, gave the proline methyl ester **17.3** in the form of an HCl salt. Meanwhile, *N*-methyl

glycine (**17.4**) was treated with PhOCOCl and K_2CO_3 in water to afford *N*-Methyl-*N*-(phenoxycarbonyl)glycine (**17.5**). This acid was then transformed into its acyl chloride **17.6** through the action of $(COCl)_2$ and a catalytic amount of DMF, and was then coupled with the *cis*-proline ester HCl salt **17.3** to give **17.7**. This experiment completed the first key step towards the construction of the diketopiperazine (DKP) ring skeleton.

Before the Dieckmann type cyclization to synthesize the DKP ring, several modifications on the ester side chain are required in order to construct the dihydrooxepin after the DKP ring is formed. To achieve this, a Swern oxidation of alcohol **17.7** using $(CF_3CO)_2O$/DMSO/Et_3N was done to yield ketone **17.8**, and the ketone was protected as a dimethyl ketal (**17.7**→**17.9**), using TsOH and $HC(OMe)_3$ in methanol.

Then, the methyl ester group of **17.9** was reduced to a primary alcohol **18.1** under mild conditions consisting of $NaBH_4/CaCl_2$. In this system, $Ca(BH_4)_2$ is formed in situ and it reduces the ester.[22] It is worth noting that the reaction temperature should be kept below –10 °C because the carbamate part of the molecule was not very stable to these reduction conditions and phenol was observed by TLC when the reaction temperature rose above –10 °C. With the alcohol **18.1** in hand, Moffat oxidation (DCC/DMSO/CF_3CO_2H/pyridine system) was selected to oxidize the alcohol, and aldehyde **18.2** was obtained in 76% yield.[23]



Scheme 18.

At this stage, the second key step of allyl group addition to aldehyde **18.2** was tested. Instead of using the normal Grignard reagent, allylmagnesium bromide, we treated allyl bromide with indium metal strips so as to form an allyl indium species in situ.[24] Addition of this allylindium species to aldehyde **18.2** gave the alcohols **18.3** in 90% yield. The diastereoselectivity was not clear

because of the existence of rotamers, which made the ^{1}H and ^{13}C NMR spectra very complicated; consequently, accurate stereochemical information could not be obtained until after the DKP ring had been formed.

Ozonolysis of the terminal alkene **18.3**, followed by reductive cleavage using $NaBH_4$, directly gave 1,3-diol **18.4**. We initially used the classic ozonolysis/Me_2S conditions to make the aldehyde and this was then reduced to the 1,3-diol **18.4**, but the yield was not very good. In another experiment, when the hydroxyl group of **18.3** was protected by reaction with AcCl (**18.5**), the ozonolysis/reduction afforded the elimination product **18.5**.

The dimethyl acetal **18.6** was then hydrolyzed to the pyrrolidinone **18.7** and the diol in **18.7** was protected using *t*-$Bu_2Si(OTf)_2$ to give **18.8**. *t*-$Bu_2Si(OTf)_2$ is much more reactive than *t*-Bu_2SiCl_2 and this protection step using *t*-Bu_2SiCl_2 was not successful.

In summary, at this stage, **18.8**, the precursor of the Dieckmann cyclization/sulfenylation step has been synthesized in 11 linear steps. We then treated **18.8** with NaH to conduct the Dieckmann cyclization, followed by treatment with a solution of Me_3SiCl/Et_3N in THF to give a silyl enol ether. Then, sulfenylation using MeSCl, which was generated in situ,[20, 25] afforded the desired bicyclic product **18.9** as a single diastereomer in 15–20% yield. The yield was disappointing, but X-ray analysis confirmed the stereochemistry of both chiral centers.

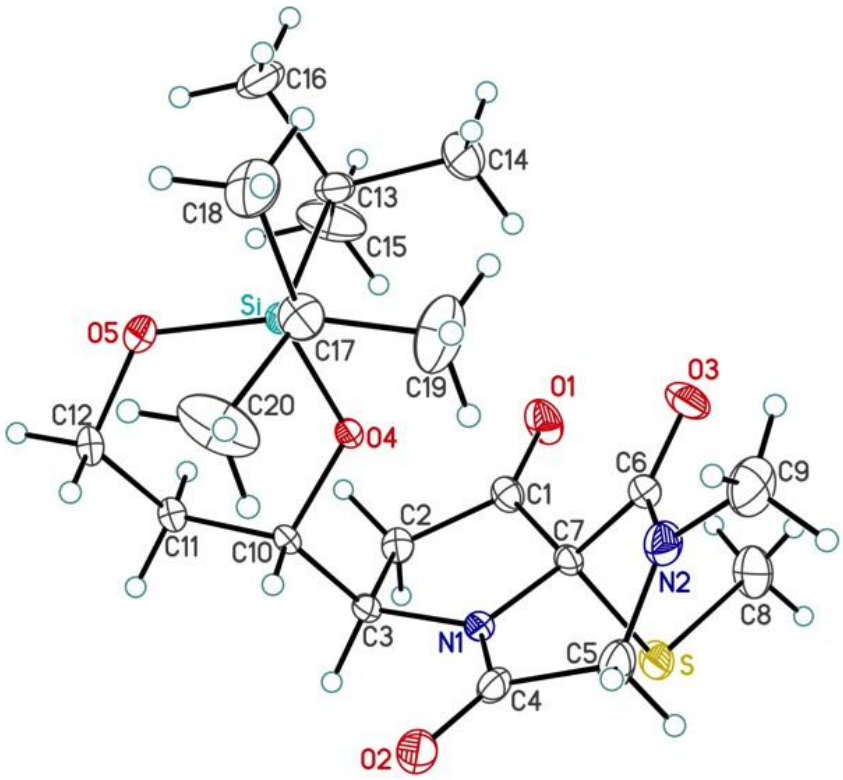
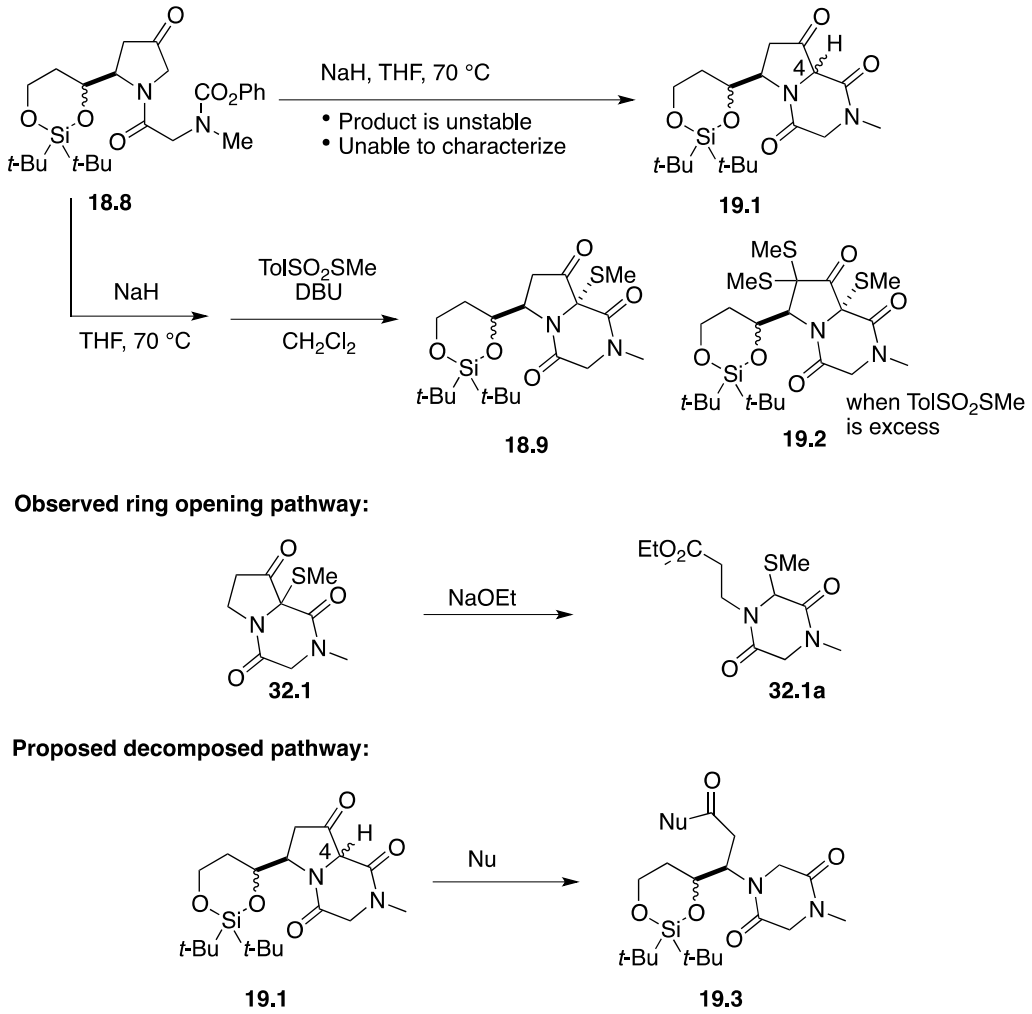


Figure 1.

The poor yield of this reaction at such a late stage of the synthesis meant that further progress could not be made. To identify the reason for the low yield and to improve this key step, we investigated the individual steps involved in the conversion of **18.8** to **18.9**.



Scheme 19.

Firstly, the Dieckmann cyclization was problematic because the product was unstable on silica and the crude NMR spectrum was also messy; consequently, we could not characterize **19.1**. Dr. J. Peng also reported similar results that his related cyclization product was not stable and partially decomposed on the silica gel.[19] The pathway of decomposition is not certain but we observed that in later studies the ring opening of **32.1** by the strong nucleophile EtONa at room temperature gave **32.1a**. Therefore, a similar

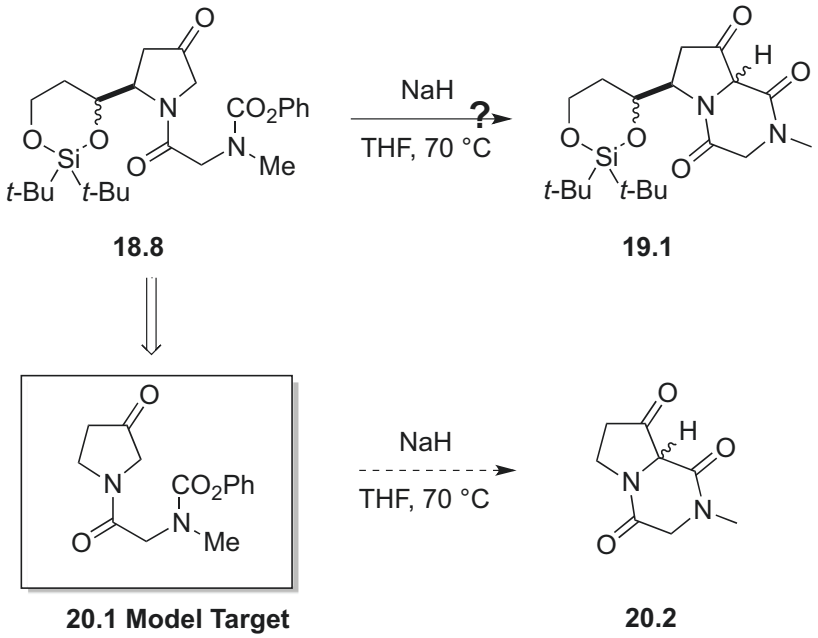
pathway can be proposed to explain the decomposition of **19.1** (**19.1**→**19.3**). While we cannot account for the stability difference between **18.9** and **19.1**, we note that the presence of the sulfur substituent blocks enolization; but how that fact confers stability is not clear.

Secondly, it is difficult to test the yield of the silyl enol ether that is formed in situ, and the efficiency of the sulfenylation is likewise difficult to measure because all the intermediates are sensitive. However, we think that the inefficiency of the first cyclization is the main reason for the low yield because we also tried the sulfenylation using DBU/TolSO_2SMe[26] after the cyclization and we got a similar poor yield. Moreover, if an excess of TolSO_2SMe was used, a trisulfenylated product **19.2** was isolated.

In order to overcome this problem and advance the synthesis, we planned to conduct model studies on the cyclization in order to find the best pathway for constructing the DKP ring.

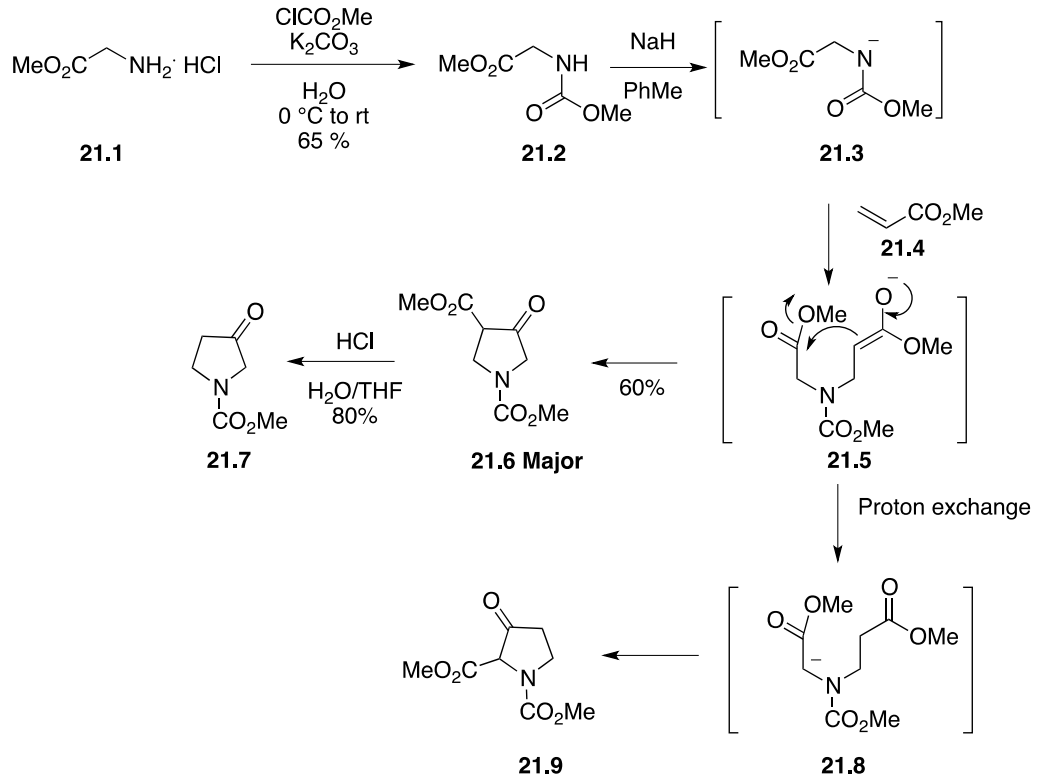
2.2 Model Studies on the DKP formation via Dieckmann cyclization

Because the Dieckmann cyclization of **18.8** did not go properly, we simplified the Dieckmann cyclization precursor and examined the model **20.1** to test the cyclization.



Scheme 20.

To make the target model, based on current literature on the synthesis of pyrrolidinones,[27] we started the synthesis with glycine methyl ester (**21.1**). Protection of the primary amine by reaction with MeOCOCl gave the carbamate **21.2** in moderate yield (65%). When **21.2** was treated with NaH, followed by addition of methyl acrylate, a Michael addition/condensation via **21.5** occurred and the desired pyrrolidinone ester **21.6** was formed as the major product. The minor undesired compound **21.9** was also formed through proton exchange and Dieckmann cyclization. Fortunately, **21.6** could be purified by recrystallization, and then acid-mediated decarboxylation gave the pyrrolidinone **21.7** in 80% yield.



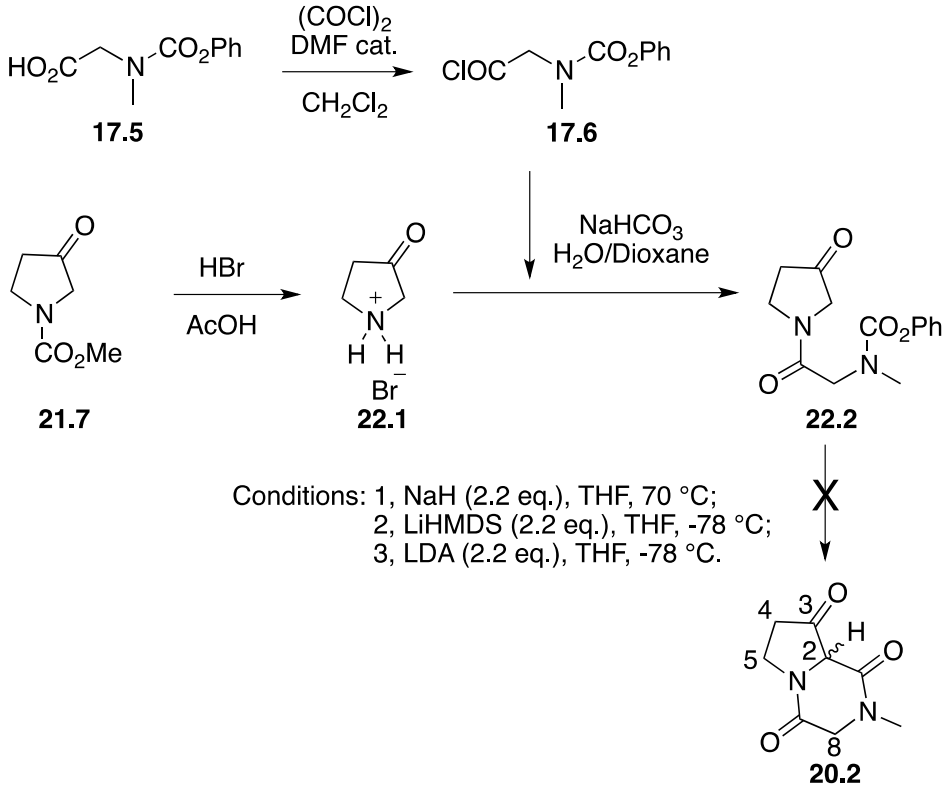
Scheme 21.

Pyrrolidinone **21.7** was deprotected with HBr in AcOH to afford pyrrolidinone HBr salt **22.1** that could be purified by crystallization.[28] The same procedure as was used for the conversion **17.3**→**17.7** was applied so as to complete the amide coupling and generate **22.2**, the model for the Dieckmann cyclization studies.

We then tested the cyclization under different basic conditions but unfortunately none of them gave the desired product. With LiHMDS or LDA at low temperature (-78 °C) the cyclization did not go to completion, and most of the starting material was recovered. Use of NaH in THF at 70 °C destroyed all the starting material even before addition of a sulfenylating reagent (Scheme 22),

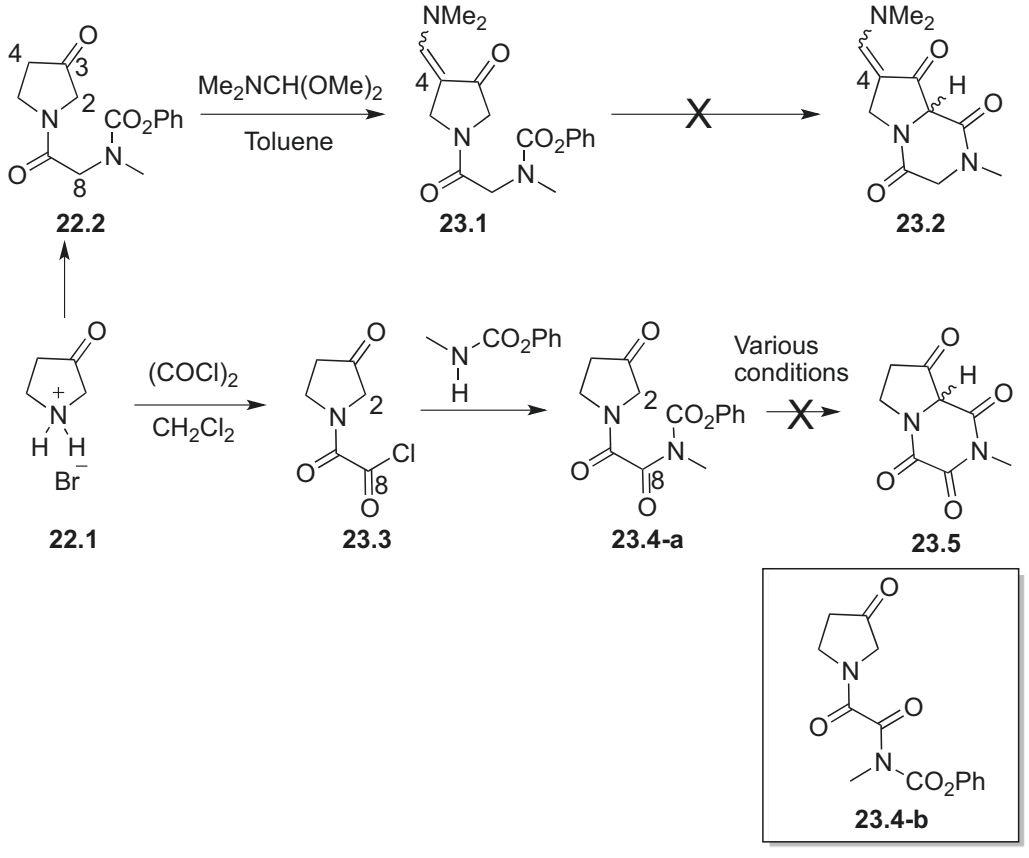
as had been observed in the attempted conversion of **18.8** to **19.1** (Scheme 19). TLC examination of the reaction mixture suggested the formation of one product, but it was unstable and decomposed, accompanied by a color change to dark brown, during workup with pH 7 buffer.

Except for the poor stability of the initial product, we also suspect that the regioselectivity of deprotonation might be another reason for the failure of this cyclization. Therefore, to increase the regioselectivity of deprotonation, we need to block the CH_2 at C(4) or C(8) (see **20.2**) and retest the cyclization.



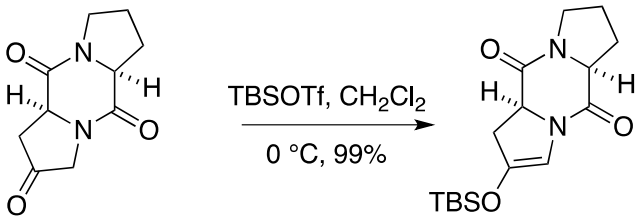
Scheme 22.

We started from **22.2** and treated it with $Me_2NCH(OMe)_2$ to produce vinylogous amide **23.1**. However, the cyclization still failed and no cyclized product **23.2** was obtained. Additionally, we also managed to block the C(8) CH_2 as follows and retest the cyclization. Compound **22.1** was reacted with $(COCl)_2$ and the resulting acyl chloride **23.3** was coupled with the protected methylamine $MeNHCO_2Ph$ to afford **23.4**. Though various basic conditions were tried, the cyclization did not go and the starting material was recovered instead. We think that the 1,2-dicarbonyl compound **23.4-a** has a preferred trans conformation **23.4-b** which is unfavorable for cyclization.



Scheme 23.

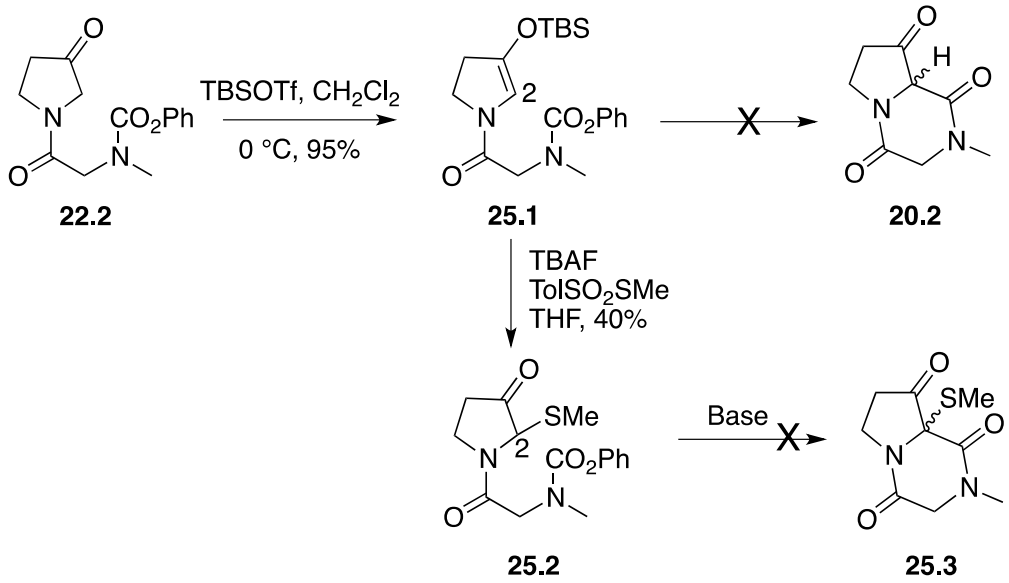
During our search for better regioselectivity in the deprotonation, we found that Fukuyama's group had published a paper on the synthesis of spirotryprostatin A and in one step they conducted a highly regioselective silyl enol ether formation (Scheme 24)[29] (**24.1**→**24.2**).



24.1 **24.2**

Scheme 24.

We tried the same conditions with **22.2** and found that silyl enol ether **25.1** was obtained in excellent yield (95%). The regioselectivity was confirmed by comparison of the ^{1}H NMR spectra of **22.2** and **25.1** (Figure 2); the chemical shifts of protons on C(4) were hardly affected.



Scheme 25.

However, Bu_4NF-mediated deprotection of the silyl enol ether did not result in cyclization. With **25.1** in hand, treatment with Bu_4NF in the presence of $TolSO_2SMe$ yielded monosulfenylated product **25.2**. In this case, theoretically, the proton on C(2) should be very acidic but deprotonation of **25.2** did not afford the cyclized product **25.3**. Therefore, we can conclude that the carbamate motif is not reactive enough towards the cyclization.

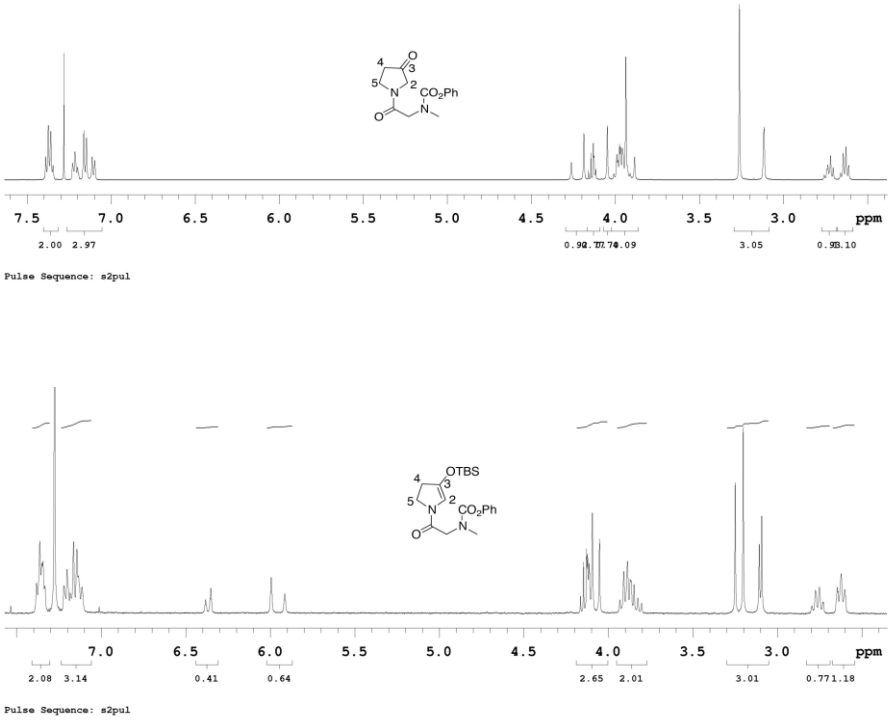
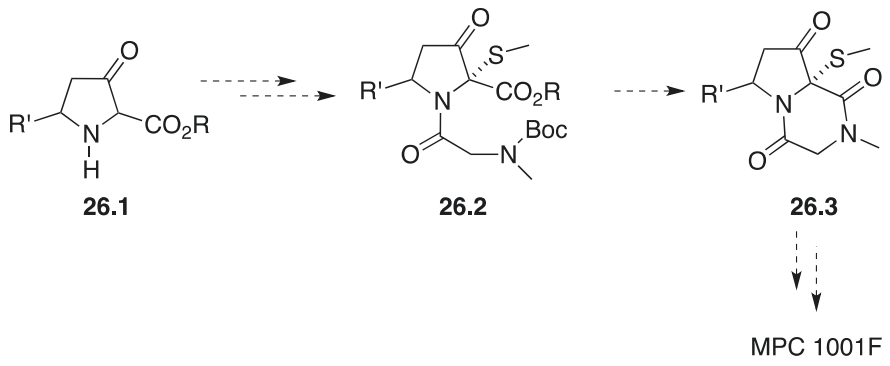


Figure 2.

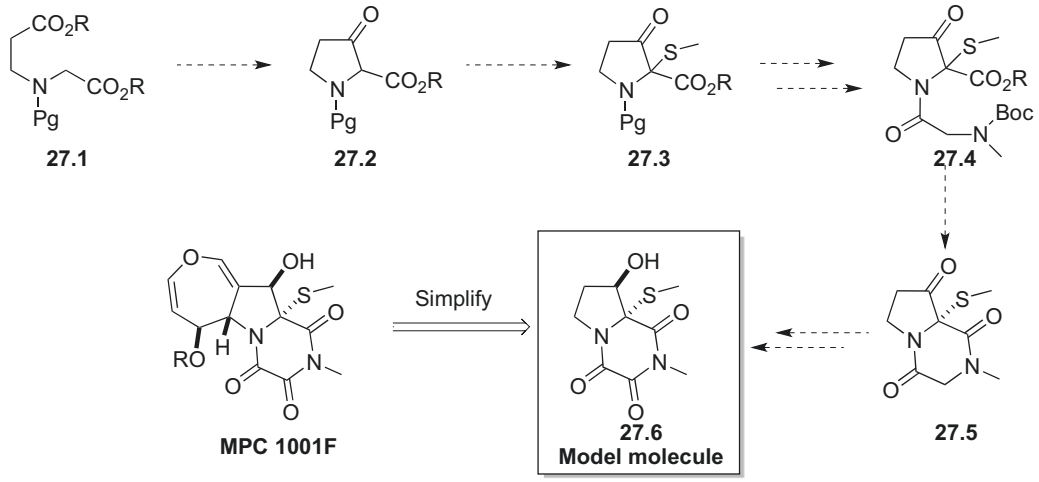
2.3 Second approach to the synthesis of MPC1001F: model studies

As the diketopiperazine (DKP) synthesis by Dieckmann type cyclization did not work well, neither on synthetic intermediate **18.8** towards MPC1001F nor on the model **22.2**, we decided to examine the ketoester **26.1** as the starting point from which to construct the DKP unit (Scheme 26).



Scheme 26.

We felt that **27.6** would be a good simplified model for MPC1001F (Scheme 27) and would serve as the preliminary synthetic target to help us test the potential challenging steps like DKP ring formation, electrophilic sulfenylation, and stereoselective pyrrolidinone reduction.

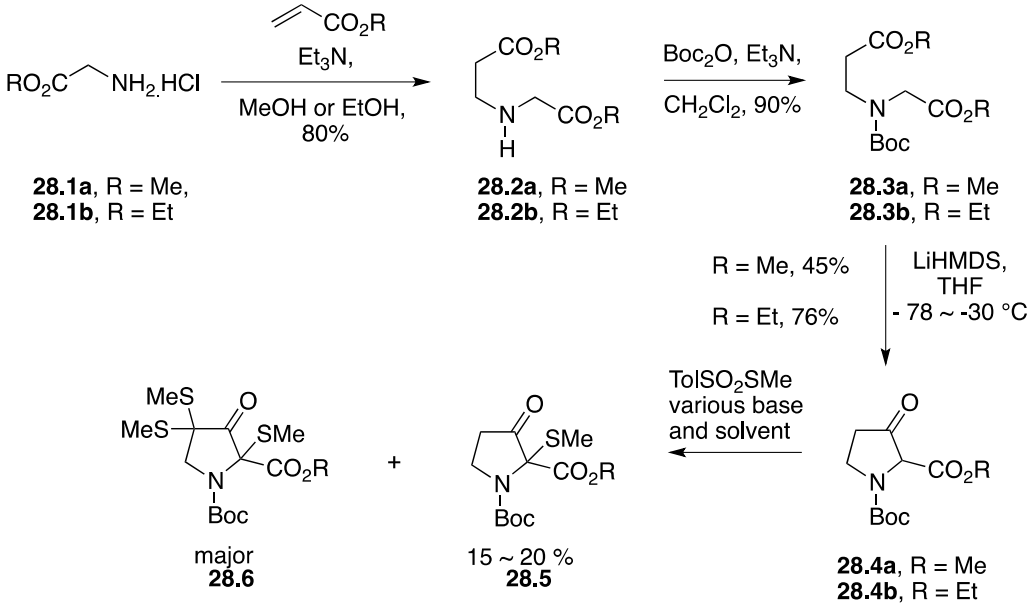


Scheme 27.

Our synthetic plan to reach the model is shown above in generic terms. The pyrrolidin-3-one **27.2** would be constructed through Dieckmann cyclization

by a known method,[30] and then the regioselective installation of the SMe group would be carried out by electrophilic sulfenylation based on the low pKa of the α-proton of the β-ketoester (**27.3**). Amine deprotection and amide coupling, followed by *N*-deprotection and intramolecular amidation, is expected to give the key intermediate **27.5**, which we hoped could be taken further by ketone reduction and stereocenter inversion, if needed.

To put these plans into practice, we started the synthesis from glycine methyl ester HCl salt (**28.1a**) and treated it with methyl acrylate and Et_3N in MeOH to afford dimethyl ester amine **28.2a**. Then Boc protection of the amine gave the precursor **28.3a** of the pyrrolidinone in 92% yield. However, Dieckmann cyclization of the dimethyl ester **28.3a** was troublesome. According to the Williams procedure,[30b] treating the starting material in THF with 1.3 eq. $(Me_3Si)_2NLi$ at –78 °C for 4 h did not give the pyrrolidinone **28.4a** in satisfactory yield (we got 48%–52%) because ca 15% of the starting material was recovered. Purification at this stage was difficult because the R_f values of the product and starting material were very similar. Fortunately, changing from a dimethyl ester to a diethyl ester (i.e. conversion of **28.1b** to **28.3b**) gave a huge improvement in the cyclization step because there was a big polarity difference between the starting material and the product, and the purification by flash chromatography was very easy.



Scheme 28.

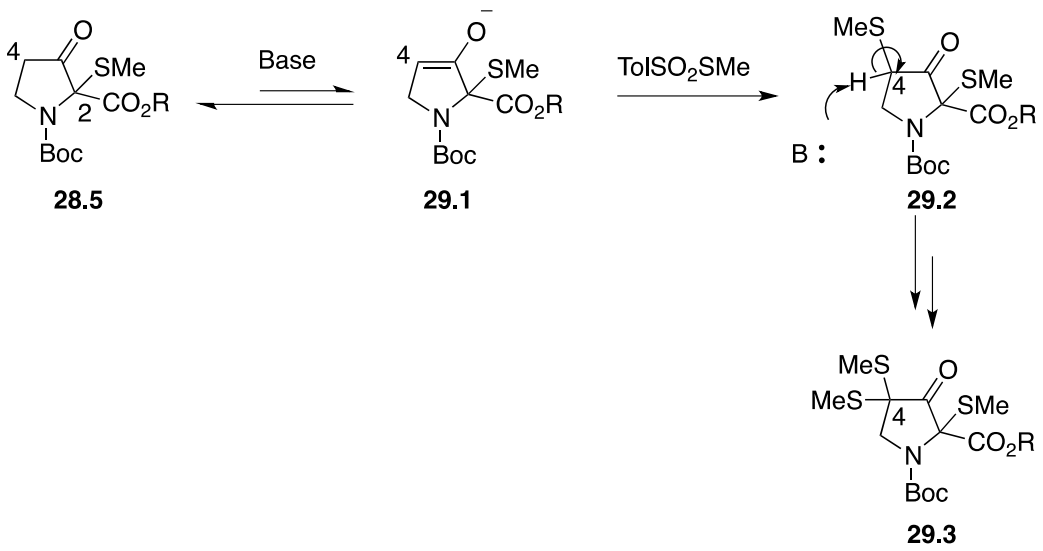
We also noticed that temperature control of this cyclization is crucial because a 4-h reaction time at –78 °C (the literature method) does not result in complete reaction, and ca 10–15% of the starting material remains. However, raising the temperature from –78 °C to –30 °C does result in complete reaction and gives the pyrrolidinone product in much better yield (76%).

At this point we used the pyrrolidin-3-one **28.4a** to test the sulfenylation. To our surprise, the major product was the trisulfenylated pyrrolidinone **28.6** and the desired monosulfenylated product **28.5** was obtained in only 15–20% yield. The 2-ethyl ester pyrrolidin-3-one **28.4b** gave almost the same result. The conditions we tried are shown in Table 2:

Table 2.

base (equivalent)	**electrophile**	**temperature**	**time**	**yield (28.5)**
DBU (1.5 eq)	$TolSO_2SMe$	r.t.	5 min	0 % (only **28.6**)
Et_3N (2.0 eq)	$TolSO_2SMe$	r.t.	6 h	20%
K_2CO_3	$TolSO_2SMe$	r.t.	6 h	15 %
pyridine (1.5 eq)	$TolSO_2SMe$	r.t.	16 h	No reaction

DBU is the strongest base we used and the reaction went to completion quickly but only the trisubstituted product **28.6** and some starting material were obtained. Weaker bases like Et_3N and K_2CO_3 gave 20% and 15% yield, respectively, as well as the major product **28.6**. Although the proton on C(2) is supposed to be the most acidic one, the protons on C(4) can evidently also be deprotonated even with weak bases. We also observed that, during the first 30 min to 1 h, only **28.5** and starting material **28.4** were present, but the trisubstituted compound **28.6** was observed with longer reaction times, and the amount of **28.5** decreased. Additionally, the trisubstituted product started to form even before the starting material had been consumed completely. As for the formation of trisulfenylated **28.6**, we suspect that equilibrium between **28.5** and **29.1** is essential. After one SMe group has been installed on C(4), the remaining proton on C(4) is much more acidic and the third sulfenylation would probably occur very rapidly.

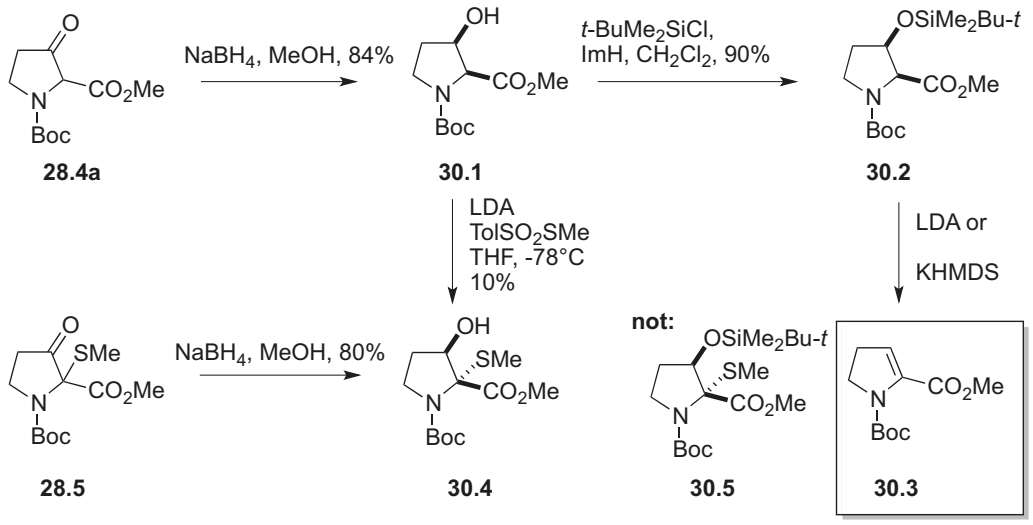


Scheme 29.

However, the reported alkylations of **28.4** do *not* show this phenomenon of over reaction[31] and the mechanism I have proposed cannot explain why the over sulfenylation does not occur from the beginning of the reaction, but only at a later stage when the starting material is still present. The kinetics of the various stages must be such that the outcome is very sensitive to concentration and it may be that the initial product is in fact acting as a reagent for sulfenylation at C(4).

To avoid this over sulfenylation, the ketone was reduced to alcohol **30.1** and the hydroxyl group was protected by reaction with *t*-butyldimethylsilyl chloride (**30.2**). However, LDA deprotonation and sulfenylation did not provide the monosulfenylated product **30.5** but the elimination product **30.3** instead. Williams's work[32] suggested that leaving the hydroxyl group unprotected would

prevent the elimination and would allow us to also install the sulfur electrophile with the desired trans stereochemistry. Unfortunately, the yield in this approach was very low (10%) and the later removal of the Boc group would probably result in loss of the SMe group.



Scheme 30.

Additionally, with compound **28.5** in hand, we also tested simple $NaBH_4$ reduction of the ketone and conveniently we found that only one diastereomer (**30.4**) was obtained which has exactly the same spectrum as the compound which was synthesized from alcohol **30.1**. Thus, the ester group in **28.5** controls the diastereoselectivity.

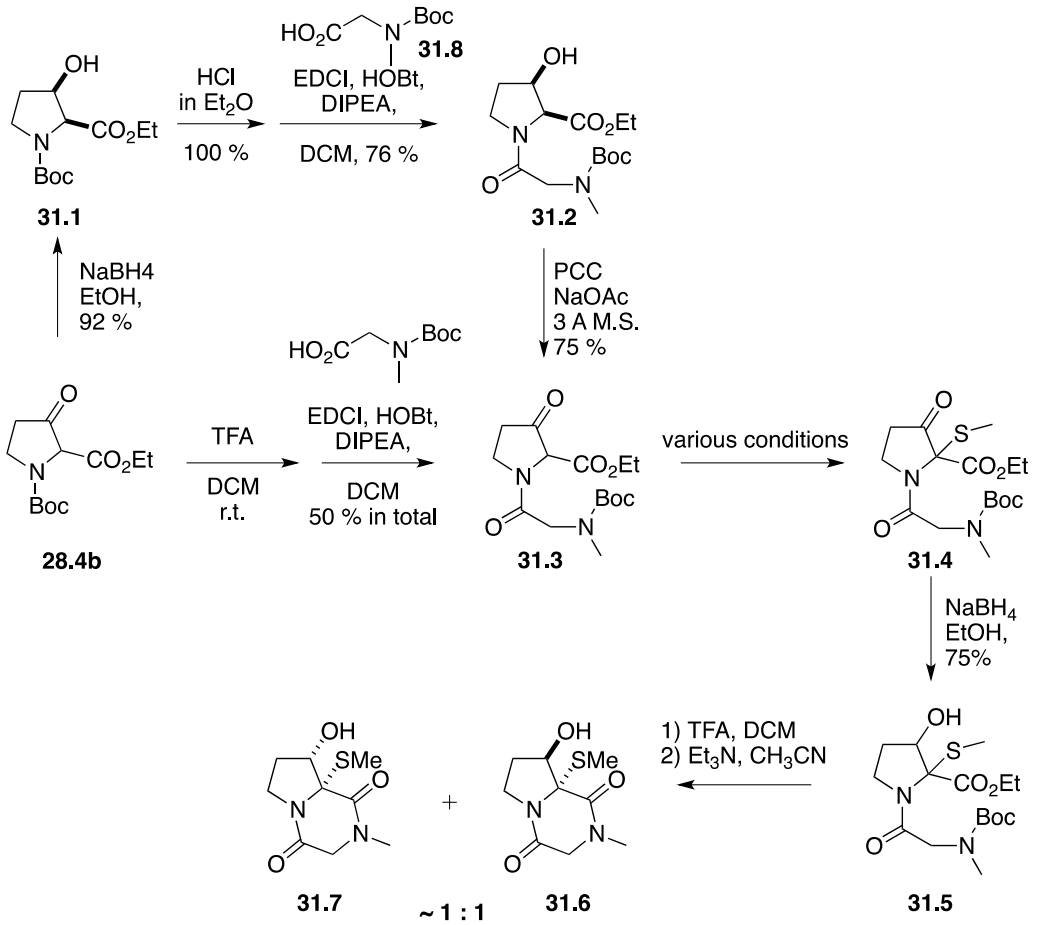
On the basis of the above observations, we can conclude that i) obtaining the monosulfenylation product is possible if we carefully control the nature of the base and the temperature; ii) the relative trans stereochemistry between the C(2) hydroxyl and the C(1) SMe group can be achieved by distereoselective

reduction; iii) the SMe group should be installed later than glycine amidation (compared with **27.3→27.4**) to avoid loss of the SMe group during the Boc deprotection and amidation steps.

Therefore, we modified the previous route and restarted our studies from **28.4b** (Scheme. 31). First, we tried to make the glycine coupled pyrrolidinone ester **31.3**. Direct amidation from deprotected pyrrolidinone **28.4b** using EDCI/DMAP gave a very poor yield (15%), but we did not stop at this stage to improve the yield. Based on a literature report,[32] we reduced the pyrrolidinone **28.4b** first and then treated the resulting **31.1** with CF_3CO_2H, followed by amidation with *N*-Boc-*N*-methyl glycine in the presence of HBTU/*i*-PrNEt; the desired compound **31.2** was obtained in 76% yield. PCC oxidation of the alcohol back to a ketone took us to the key intermediate **31.3**.

Considering the high price of HBTU, we tried to use EDCI/*i*-PrNEt/HOBt to achieve the amidation. However, the yield was much lower than with HBTU. To solve this, we planned to purify the deprotection product and isolate the pure amine. Therefore, instead of using CF_3CO_2H, we used commercial HCl in Et_2O[33] to remove the Boc group, and the resulting amine HCl salt was obtained as a solid in almost quantitative yield. Amidation between the amine HCl salt and glycine derivative **31.8** in the presence of *i*-PrNEt/EDCI/HOBt gave the product **31.2** in reasonable yield (75–78%) without the need for chromatography. Having these modified conditions established, we went back to pyrrolidinone **28.4b** and repeated the direct deprotection-amidation reaction, and finally, the desired product was synthesized in 50% yield overall

from **28.4b**, which is higher than the overall yield of the previous route (**28.4b→31.1→31.2→31.3**)(Scheme 31).



Scheme 31.

After solving a series of problems in the preparation of the starting material **31.3**, we investigated some more conditions (Table 3) to achieve the monosulfenylation. Finally, we found that treating **31.3** with 1.0 eq. Cs_2CO_3 and 1.0 eq. $TolSO_2SMe$ at –20 °C in MeCN largely prevented the formation of over

sulfenylated compounds and gave the desired product **31.4** in 52% absolute yield (or 74% corrected for recovered starting material).

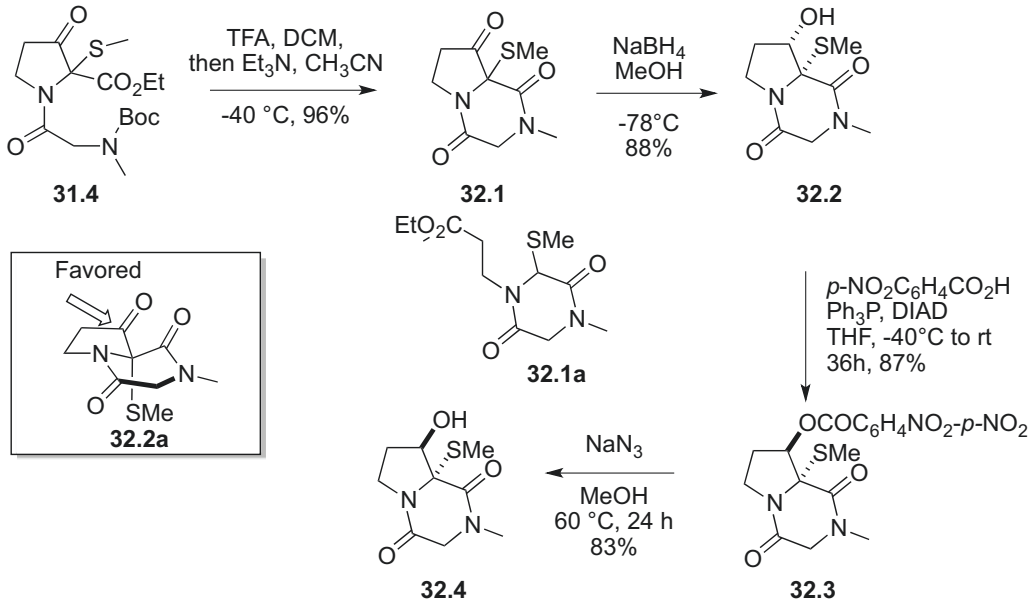
Table 3.

base (equivalent)	electrophile	temperature	Solvent	time	yield
DBU (1.3 eq.)	$TolSO_2SMe$ (1.3 eq.)	- 78 °C	CH_2Cl_2	45 min	Over sulfenylation occurred quickly
DMAP (1.3 eq.)	$TolSO_2SMe$ (1.3 eq.)	r.t.	CH_2Cl_2	24 h	Very slow and trace DP observed
DMAP (1.3 eq.)	$TolSO_2SMe$ (1.3 eq.)	40 °C	CH_2Cl_2	48 h	20% DP without over sulfenylation
Cs_2CO_3 (1.3 eq.)	$TolSO_2SMe$ (1.3 eq.)	- 20 °C	MeCN	1.5 h	40% (68% recovered)
Cs_2CO_3 (1.0 eq.)	$TolSO_2SMe$ (1.0 eq.)	- 20 °C	MeCN	1.5 h	52% (74% recovered)

After reaching the mono-sulfenylated compound **31.4**, $NaBH_4$ reduction gave the desired alcohol **31.5**. The relative stereochemistry between the hydroxyl and SMe group at this stage was not clear from NMR spectra due to the existence of rotamers. However, *N*-Boc deprotection with CF_3CO_2H as well as DKP ring formation gave two isomers in a ratio of about 1:1. Evidently, the reduction of ketone **31.4** did not give the expected single diastereomer **31.5** as was the case for the conversion of **28.5** to **30.4**. The reason for this behavior is not known but possibly the flexibility of the glycine derived side chain neutralizes any inherent facial bias imposed by the SMe and CO_2Me groups.

To simplify the problem of this undesired nonselective ketone reduction, we carried out the deprotection and cyclization from compound **31.4** and

obtained the bicyclic compound **32.1** in 96% yield. In this reaction, compound **32.1a** was isolated when the reaction temperature was higher than -10 °C because the leaving EtO^- group can attack the ketone of the pyrrolidinone **32.1** and open the ring. Reduction of **32.1** with $NaBH_4$, now afforded the cis product **32.2**. At this point, Mitsunobu reaction smoothly inverted the stereochemistry of the hydroxyl group (**32.2**). It is worth noting that the concentration in the Mitsunobu reaction influenced the rate of the reaction dramatically and an ideal concentration should be higher than 0.1 M.



Scheme 32.

At this stage, a 1D TROESY experiment (Figure 3) clearly showed the correlation between the SMe group and the proton on C(3), which strongly confirmed the desired trans stereochemistry. Hydrolysis of **32.3** using unusual

conditions (NaN_3 in MeOH)[34] gave the free alcohol **32.4** and the relative stereochemistry was again confirmed by a 1D TROESY experiment.

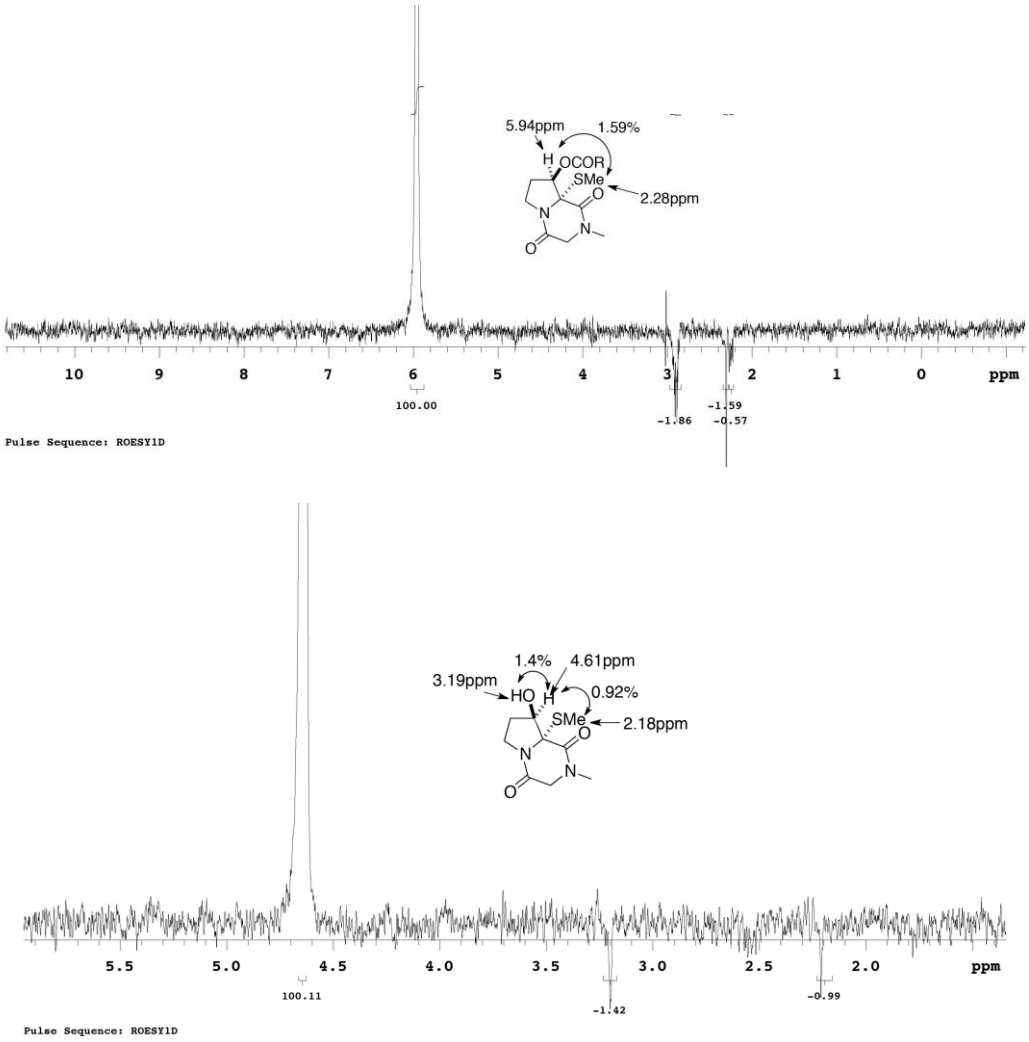
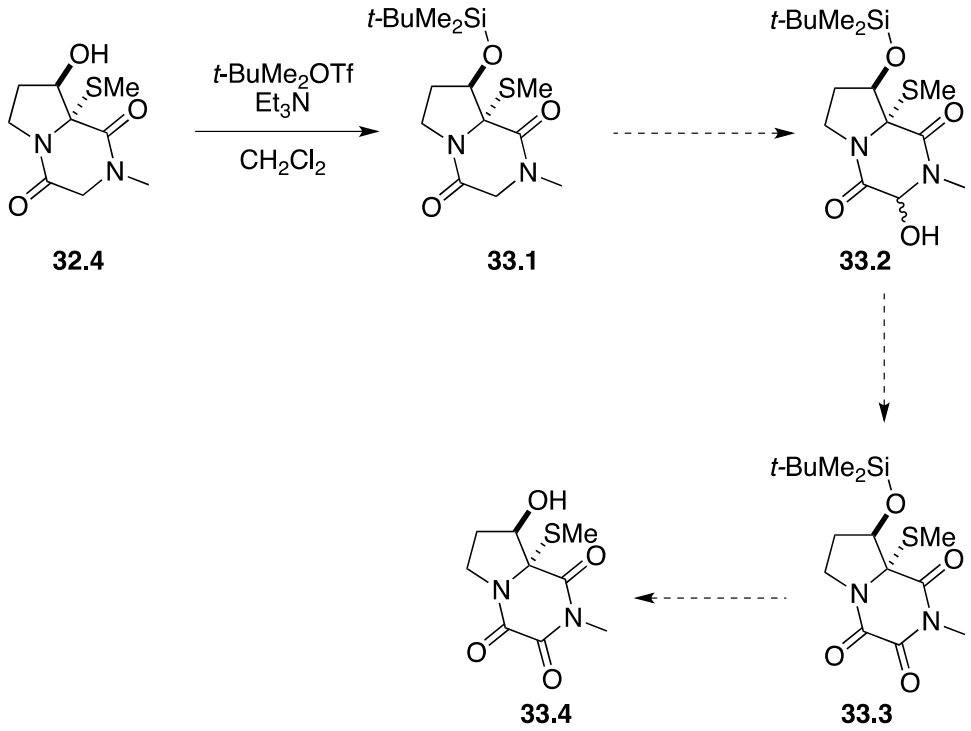


Figure 3.

At this stage, we have successfully solved the challenging problems of DKP ring formation, sulfenylation and setting the trans stereochemistry between the hydroxyl and SMe groups.

As we are studying the total synthesis of MPC1001F, we now had to solve the last problem of installing the third C=O bond on the DKP ring.

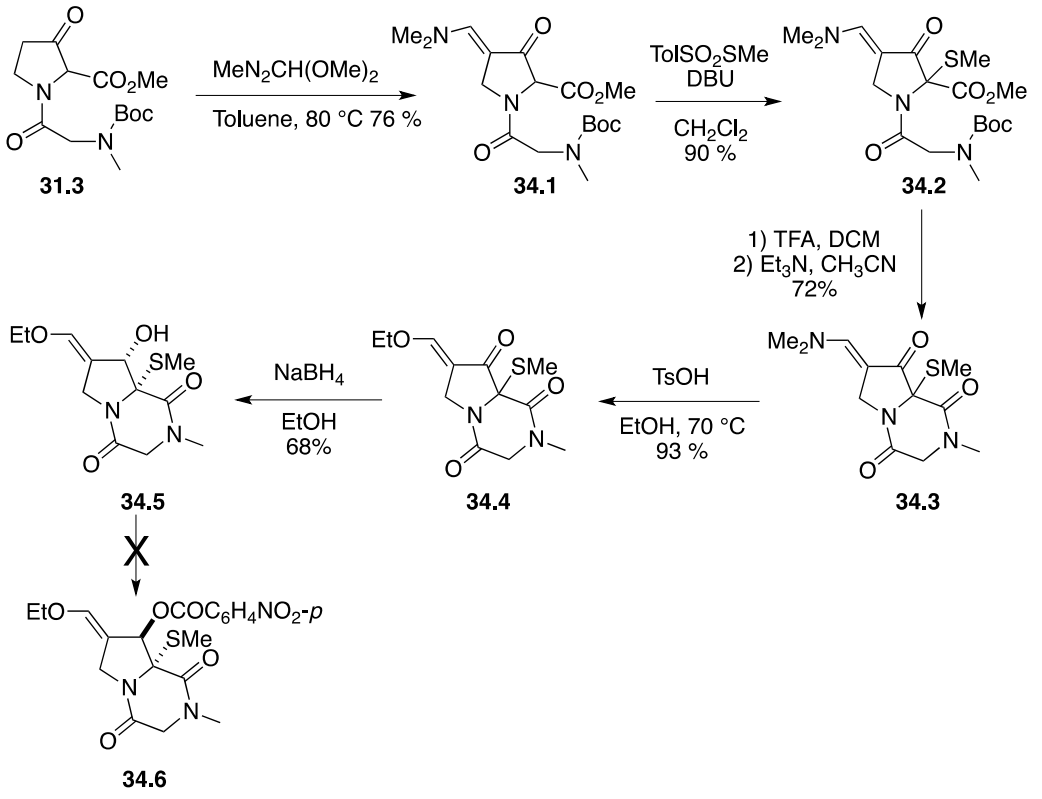
We first protected the hydroxyl group as a *t*-butyldimethylsilyl ether by reaction with *t*-$BuMe_2SiOTf/Et_3N$ (84%); the classic *t*-$BuMe_2SiCl$/imidazole conditions did not work. As of this writing we have reached compound **33.1** and its hydroxylation will be studied in the future.



Scheme 33.

During previous studies on the synthesis of MPC molecules, our laboratory developed a method to construct the oxepin skeleton (Scheme. 10).[12] Therefore, we decided to install the (dimethylamino)methylene group on the pyrrolidinone **31.3** for the convenience of oxepin ring formation at a later stage;

the presence of this unit would also prevent over sulfenylation. We treated **31.3** with DMF-DMA in refluxed toluene and **34.1** was obtained in good yield.[35] The sulfenylation then went smoothly and afforded the monosulfenylated **34.2** in 90%. Deprotection of **34.2**, followed by intramolecular amidation, gave the bicyclic compound **34.3** and a 1D TROSEY experiment confirmed the alkene geometry. With a view to constructing the oxepin ring in future work, we treated the **34.3** with TsOH in EtOH, and **34.4** was formed to afford a molecular skeleton resembling a significant part of MPC1001F.

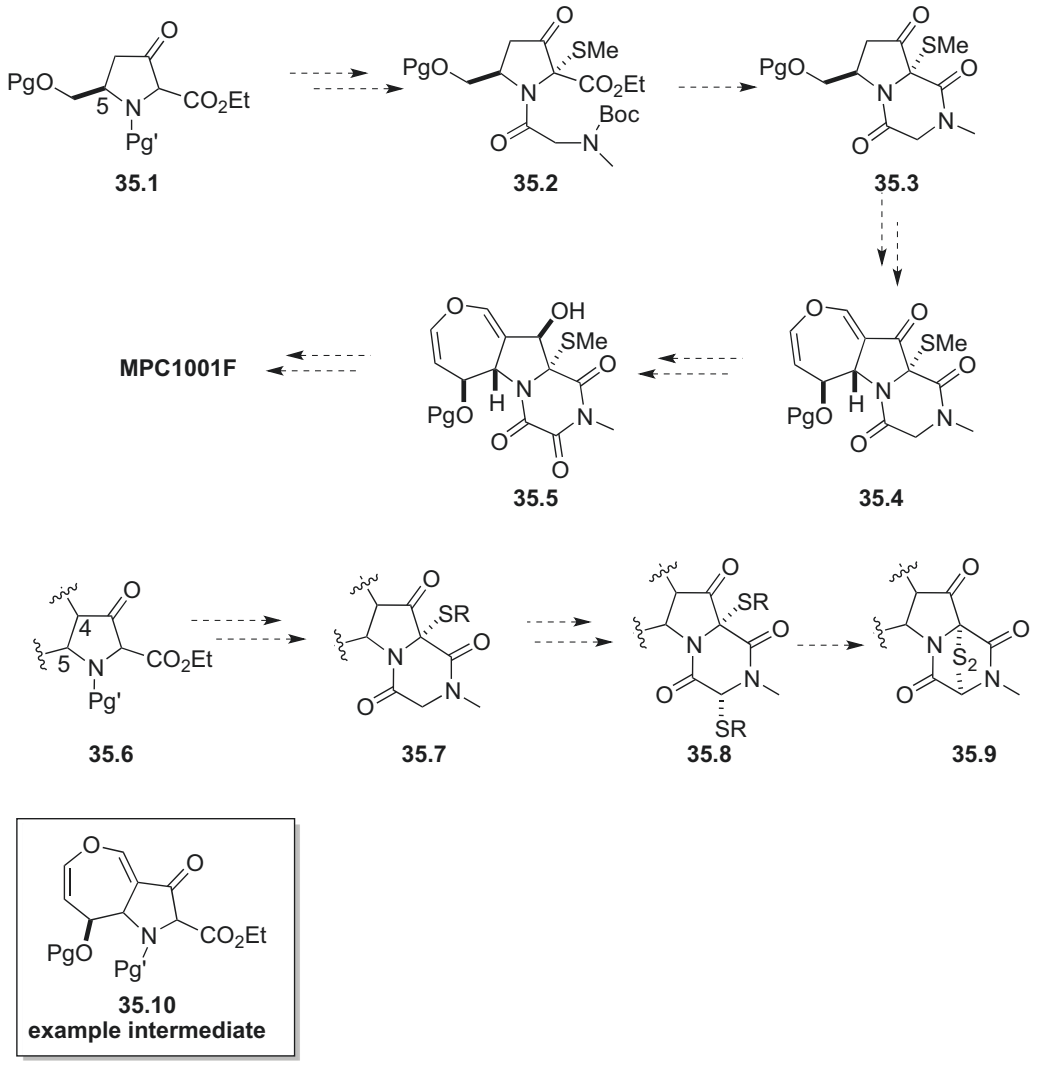


Scheme 34.

We continued our studies with **34.4** and found that $NaBH_4$ reduction also gave the cis product **34.5**. Although our attempts at stereocenter inversion via Mitsunobu reaction did not work, probably due to the rearrangement possibilities of the allyl ether part and the electron donating effect from the ethoxy group, this route is still valuable because the electron donating effect of the oxygen in the dihydrooxepin ring of the MPC1001F itself is lower than in our ethoxy model compound **34.5** and the stereocenter inversion should be easier because the oxepin oxygen is flanked on *both* sides by a carbon-carbon double bond.

2.4 Future plans

With this general and robust route to the BC ring system of MPC1001F, in the future, we need to introduce an extra oxygen on ring C and build the dihydrooxepin ring to complete the synthesis of the ABC core structure **35.5** of the MPC1001F.



Scheme 35.

Therefore, compound **35.1** will be a suitable starting material and the sulfenylated **35.2** should be reached easily with the same route created in the model studies. Then the DKP ring formation would give the BC ring skeleton. This laboratory has already developed the method to construct the dihydrooxepin ring based on the intermediates of type **35.3**[12] and to make the disulfide bridge[36]

and we believe that we have gained valuable experience in solving the potential challenges towards the MPC1001F and MPC1001.

Alternatively, as the ETP type natural products usually have the same BC ring system as MPC1001 natural products but a different substructure on the other side of the pyrrolidine ring, the C(4), C(5) functionalized intermediate **35.6** might give us access to other ETP natural products like gliotoxin **1.2** and rostratin **1.3** and acetylaranotin **1.4**.

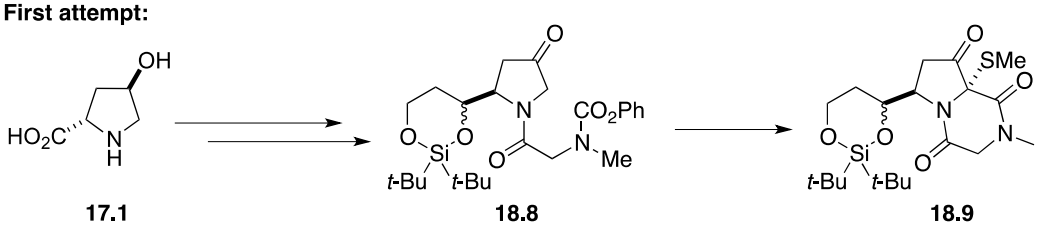
3 Conclusion

In this thesis, the first attempt to synthesize MPC1001F from naturally occurring proline **17.1** could not be taken to completion. But the 3D structure of bicyclic DKP compound **18.9** gave us much information about the conformation and stereoselectivity in our model studies.

The studies on the DKP ring synthesis via Dieckmann cyclization did not achieve the desired pyrrolidinone fused diketopiperazine skeleton (e.g. **20.2**) even though the regioselectivity of deprotection had been controlled (**25.1**→**20.2**); this illustrated that the carbamate motif is not reactive enough towards the condensation.

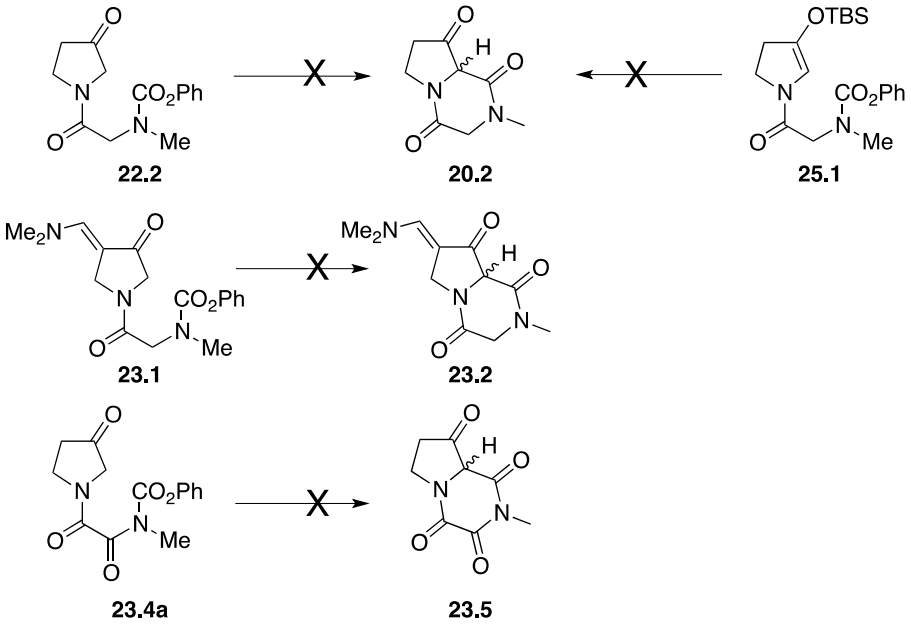
Finally, we developed a new route to the BC ring system from the pyrrolidin-3-one **28.4b** and the model **32.4** was synthesized. Compared with other ETP natural product syntheses, this route has its own highlights: i) the C(3) hydroxyl group is obtained directly from a pyrrolidinone; ii) we use electrophilic sulfenylation to install the SMe group which is easy and efficient compared with nucleophilic attack using H_2S or MeSH; iii) the trans stereochemistry between the hydroxyl and SMe groups can be set via Mitsunobu reaction and to our knowledge, this is the first application of this strategy in ETP natural products synthesis.

First attempt:



17.1 **18.8** **18.9**

Studies towards the Dieckmann cyclization:

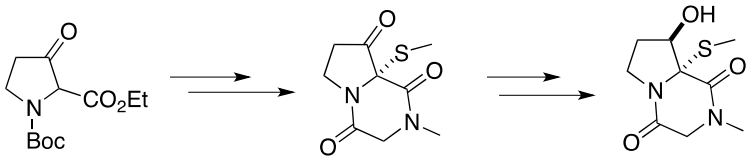


22.2 **20.2** **25.1**

23.1 **23.2**

23.4a **23.5**

New route towards the BC ring of MPC1001F:



28.4b **32.1** **32.4**

Scheme 36.

4 Experimental Section

General Procedures. Solvents used for chromatography were distilled before use. Commercial thin layer chromatography plates (silica gel, Merck 60F-254) were used. Silica gel for flash chromatography was Merck type 60 (230–400 mesh). Dry solvents were prepared under an inert atmosphere and transferred by syringe or cannula. The symbols s, d, t and q used for ^{13}C NMR spectra indicate zero, one, two, or three attached hydrogens, respectively, the assignments being made from APT spectra. Solutions were evaporated under water pump vacuum and the residue was then kept under oil pump vacuum. High resolution electrospray mass spectrometric analyses were done with an orthogonal time of flight analyzer and electron ionization mass spectra were measured with a double-focusing sector mass spectrometer.

(8a*R*)-6-[(4*S*)-2,2-di-*tert*-Butyl-1,3,2-dioxasilan-4-yl]-2-methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4,8-trione (18.9).

18.8 **18.9**

NaH (8 mg, 60 %, w/w, 0.18 mmol) was added to a stirred solution of **18.8** (39.2 mg, 0.08 mmol) in THF (4 mL) and the resulting mixture was

lowered to a preheated (70 °C) oil bath and the stirring was continued for 15 min. The reaction mixture was cooled to room temperature and then to 0 °C.

In another round bottom flask, under N_2 protection, Me_3SiCl (60 □L, 0.48 mmol) was added to a stirred and cooled (0 °C) solution of Et_3N (50 □L, 0.32 mmol) in THF (2 mL) and the stirring was continued for 30 min. 1 mL of this Et_3N-Me_3SiCl solution was then added to above NaH reaction system and the resulting mixture was stirred at 0 °C for 4 h to make silyl enol ether.

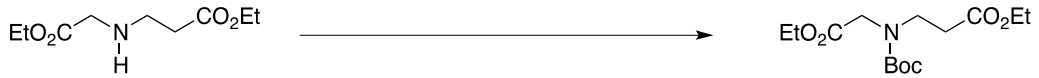
In another round bottom flask, SO_2Cl_2 (146 □L, 1.8 mmol) was added to a stirred and cooled (- 78 °C) solution of Me_2S_2 (162 □L, 1.8 mmol) in CH_2Cl_2 (5 mL). After 15 min, 0.25 mL of this solution was added to above silyl enol ether mixture at – 78 °C. Cooling bath was left but recharged and the stirring was continued overnight. Solvent was evaporated and residue was partitioned with water and CH_2Cl_2. The organic layer was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (0.5 × 12 cm), using EtOAc/CH_2Cl_2 (1:5), gave 18.9 (7 mg, 20 %) as a foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (s) and 0.95 (s, 18 H), 1.56 (s, 2 H), 1.70–1.82 (m, 1 H), 2.26 (s, 3 H), 2.62 (d, *J* = 17.6 Hz, 1 H), 3.00 (s, 3 H), 3.22 (dd, *J* = 17.6, 9.6 Hz, 1 H), 3.80 (d, *J* = 16.8 Hz, 1 H), 4.13–4.16 (m, 2 H), 4.33 (d, *J* = 9.2 Hz, 1 H), 4.56 (d, *J* = 16.8 Hz, 1H), 4.78 (d, *J* = 11.6 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (q), 20.0 (s), 22.6 (s), 26.7 (q), 27.5 (q), 32.3 (t), 32.5 (t), 33.8 (q), 53.4 (t), 56.6 (d), 60.9 (s), 64.1 (t), 71.2 (d), 160.3 (s), 165.7 (s), 193.8 (s); exact mass (electrospray) *m*/*z* calcd for $C_{20}H_{34}N_2NaO_5SSi$ $(M + Na)^+$ 442.1958, found 442.1952.

Ethyl 3-[(2-Ethoxy-2-oxoethyl)amino]propanoate (28.2b).

28.1b **28.2b**

Ethyl acrylate (9.7 g, 97 mmol) and Et_3N (21 mL, 97 mmol) were added to a stirred solution of glycine ethyl ester hydrochloride **28.1b** (20 g, 145 mmol) in EtOH (95%, 160 mL). The resulting solution was stirred at room temperature for 48 h and then evaporated. The residue was partitioned between EtOAc (80 mL) and water (80 mL) and the aqueous phase was extracted with EtOAc (2 × 60 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$) and evaporated. Distillation of the residue (1.5 Torr, 104-108 °C) gave **28.2b** (17 g, 76%) as a colorless liquid: FTIR ($CDCl_3$, cast) 3345, 2982, 1736, 1189 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.26 (q, 6 H), 1.76 (br, 1 H), 2.49 (t, *J* = 7.0 Hz, 2 H), 2.89 (t, *J* = 7.0 Hz, 2 H), 3.40 (s, 2 H), 4.14 (q, *J* = 7.0 Hz, 2 H), 4.18 (q, *J* = 7.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.3 (q), 14.4 (q), 35.1 (t), 44.9 (t), 51.1 (t), 60.6 (t), 60.9 (t), 172.4 (s), 172.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_9H_{17}NNaO_4$ $(M + Na)^+$ 204.1230, found 204.1233.

Ethyl 3-{[(*tert*-Butoxy)carbonyl](2-ethoxy-2-oxoethyl)amino}propanoate (28.3b).



28.2b **28.3b**

Boc_2O (22 g, 101.26 mmol) was added to a stirred and cooled (0 °C) solution of **28.2b** (15 g, 73.8 mmol) in $CHCl_3$ (150 mL). An aqueous solution of NaOH (8.7 g in 150 mL in water) was then added over 5 min, the ice bath was removed and stirring was continued overnight. The aqueous portion of the reaction mixture was extracted with $CHCl_3$ (50 mL) and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. Distillation of the residue (0.5 Torr, 118-120 °C) gave **28.3b** (19 g, 85 %) as a colorless liquid which was a mixture of rotamers: FTIR ($CDCl_3$, cast) 2980, 2936, 1749, 1735, 1703 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.23–1.28 (m, 6 H), 1.41 (s, 4.5 H, rotamer), 1.47 (s, 4.5 H, rotamer), 2.59–2.65 (dt, *J* = 15.5 Hz, 6.5 Hz, 2 H), 3.51–3.58 (dt, *J* = 15.5 Hz, 6.5 Hz, 2 H), 3.94 (s, 1 H), 4.01 (s, 1 H), 4.11–4.19 (dq, *J* = 27.5 Hz, 7.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q) and 14.2 (q), 14.2 (q) and 14.3 (q), 28.2 (q) and 28.3 (q), 33.8 (t) and 34.2 (t), 44.8 (t) and 44.9 (t), 50.1 (t) and 50.9 (t), 60.5 (t) and 60.6 (t), 60.9 (t) and 61.0 (t), 80.4 (s) and 80.5 (s), 155.0 (s), 155.5 (s), 170.1 (s), 170.3 (s), 172.1 (s), 172.4 (s); exact mass (electrospray) *m*/*z* calcd for $C_{14}H_{25}NNaO_6$ $(M + Na)^+$ 326.1574, found 326.1579.

(±)-3-Oxopyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-ethyl ester (28.4b).

28.3b **28.4b**

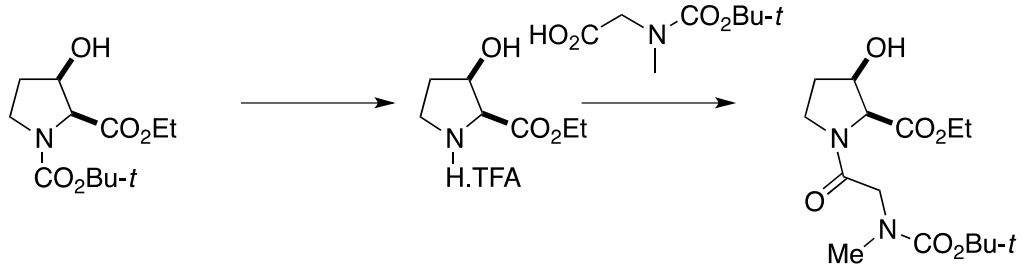
A solution of **28.3b** in THF (6 mL) was added dropwise over 5 min to a stirred and cooled (–78 °C) solution of $(Me_3Si)_2NLi$ (1.0 M in THF, 10 mL, 10 mmol) in THF (60 mL). The cold bath was left in place, but not recharged, and stirring was continued for 4 h, by which time the temperature had risen to –30 °C. The mixture was poured into cooled (0 °C) dilute hydrochloric acid (1 M, 100 mL) (swirling) and extracted with 3 portions of EtOAc. The combined organic extracts were washed twice with pH 9.5 buffer and then with brine, dried ($MgSO_4$) and evaporated to give **28.4b** (1.2 g, 71%) as a pale yellow oil which was pure enough for the next stage. The material had: FTIR ($CDCl_3$, cast) 2980, 2934, 1774, 1742, 1708, 1394, 1194 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ □□□□ □t, *J* = 6.8 Hz, 3 H□□□□.□□□ (s) □and□□□□□□□s, 9 H), 2.68 (t, *J* = 7.6 Hz, 2 H), 3.76–3.89 (m, 2 H), 4.21–4.25 (m, 2 H), 4.46 (s) and 4.55 (s, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q) and 14.2 (q), 28.2 (q) and 28.3 (q), 36.5 (t) and 37.2 (t), 41.6 (t) and 42.3 (t), 62.2 (t) and 62.3 (t), 65.5 (d) and 65.8 (d), 81.1 (s) and 81.2 (s), 153.9 (s), 166.2 (s), 204.7 (s); exact mass (electrospray) *m*/*z* calcd for $C_{12}H_{19}NNaO_5$ $(M + Na)^+$ 280.1155, found 280.1159.

1-*tert*-Butyl 2-Ethyl (±)-*cis*-3-hydroxypyrrolidine-1,2-dicarboxylate (31.1).

28.4b **31.1**

$NaBH_4$ (0.7 g, 18.7 mmol) was added in one portion to a stirred and cooled (0 °C) solution of **28.4b** (4.0 g, 15.56 mmol) in EtOH (60 mL). The ice bath was left in place, but not recharged, and stirring was continued for 1 h, by which time the mixture had attained room temp. The solution was recooled to 0 °C and saturated aqueous NH_4Cl solution (5 mL) and water (45 mL) were added. The aqueous phase was extracted with EtOAc (3 × 45 mL) and the combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (3.5 × 15 cm), using EtOAc, gave **31.1** (3.5 g, 88 %) as a colorless oil: FTIR ($CDCl_3$, cast) 3439, 2979, 1744, 1702, 1677, 1395, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ □□□□–□□□□ □m, 3 H), 1.41 (s) and 1.46 (s, 9 H), 1.99–2.11 (m, 2 H), 2.23–2.40 (m, 1 H), 3.48–3.70 (m, 2 H), 4.19–4.45 (m, 3 H), 4.56–4.61 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ □□□2 (q) □and 14.4 (q), 28.2 (q) and 28.4 (q), 32.3 (t) and 32.9 (t), 43.8 (t) and 44.3 (t), 61.2 (t) and 61.3 (t), 63.4 (d) and 63.9 (d), 71.5 (d) and 72.5 (d), 80.1 (s) and 80.2 (s), 153.8 (s), 170.4 (s); exact mass (electrospray) *m*/*z* calcd for $C_{12}H_{21}NNaO_5$ $(M + Na)^+$ 282.1312, found 282.1312.

Ethyl (±)-*cis*-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-3-hydroxypyrrolidine-2-carboxylate (31.2).

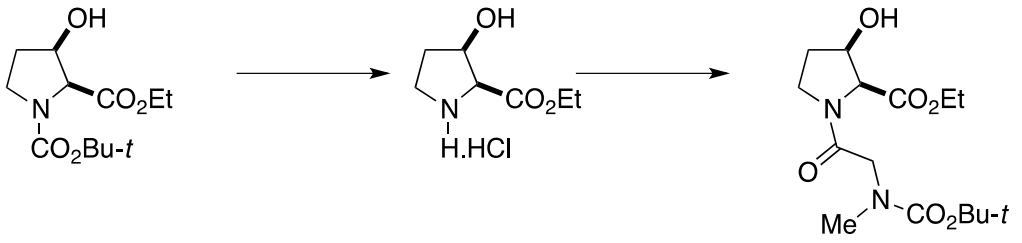


31.1 **31.2**

CF_3CO_2H (7 mL) was added to a stirred and cooled (0 °C) solution of **31.1** (730 mg, 2.8 mmol) in CH_2Cl_2 (35 mL) and after 10 min the cooling bath was removed and stirring was continued for 3 h. The CH_2Cl_2 and excess of CF_3CO_2H were evaporated and the residue was dissolved in CH_2Cl_2 (30 mL). The resulting solution was stirred and *i*-Pr_2NEt (1.5 mL, 8.4 mmol), HBTU (1.27 g, 3.36 mmol) and acid **31.8** (530 mg, 2.8 mmol) were then added in that order. Stirring was continued overnight and the mixture was quenched with dilute hydrochloric acid (1 M, 30 mL). The organic phase was washed by brine (30 mL), dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 × 15 cm), using 1:1 EtOAc-hexane, gave **31.2** (535 mg, 56 %) as a foam: FTIR ($CDCl_3$, cast) 3416, 2977, 2923, 1742, 1699, 1664 cm^{-1}; ^1H NMR ($CDCl_3$, 500 Hz) δ 1.22–1.38 (m, 3 H), 1.42 (s) and 1.45 (s, 9 H), 2.10–2.20 (m, 2 H), 2.28–2.41 (m, 1 H), 2.95 (s, 3 H), 3.40–3.78 (m, 2 H), 3.82–4.05 (m, 1 H), 4.15–4.32 (m, 2 H), 4.55–4.65 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 14.2 (q)

and 14.3 (q), 28.3 (q) and 28.4 (q), 30.8 (t) and 33.5 (t), 35.4 (q), 44.1 (t), 50.6 (t), 61.5 (t), 63.5 (d) and 63.7 (d), 70.4 (d) and 73.0 (d), 80.1 (s), 156.2 (s), 168.1 (s), 169.2 (s); exact mass (electrospray) *m*/*z* calcd for $C_{15}H_{26}N_2NaO_6$ $(M + Na)^+$ 353.1683, found 353.1682.

Ethyl (±)-*cis*-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-3-hydroxypyrrolidine-2-carboxylate (31.2). The intermediate salt is **Ethyl (±)-*cis*-3-hydroxypyrrolidine-2-carboxylate hydrochloride (DP-1).**



31.1 **DP-1** **31.2**

A solution of HCl in Et_2O (2 M, 12 mL, 24 mmol) was added to a stirred solution of **31.1** (1.5 g, 5.79 mmol) in CH_2Cl_2 (80 mL) and stirring at room temperature was continued overnight. Evaporation of the solvent left a white solid which was dissolved in the minimum amount of dry MeOH at 0 °C. Dry Et_2O was added and the white precipitate was filtered off and dried in vacuo to give **DP-1** (1 g, 98 %).

i-Pr_2NEt (0.78 mL, 4.48 mmol) was added to a stirred and cooled (0 °C) solution of **DP-1** (0.5 g, 2.56 mmol) and acid **31.8** (0.44 g, 2.30 mmol) in CH_2Cl_2 (30 mL). After 15 min HOBt (0.414 g, 3.07 mmol) and EDCI (0.476 g,

3.07 mmol) were added sequentially. The cold bath was removed and stirring was continued for 16 h. Saturated aqueous $NaHCO_3$ solution (50 mL) was added and the organic phase was washed with dilute hydrochloric acid (1 M, 40 mL), dried ($MgSO_4$), and evaporated to give **31.2** (0.62 g, 75 %) as a foam which was used without further purification: **compound data is the same as the previous one**

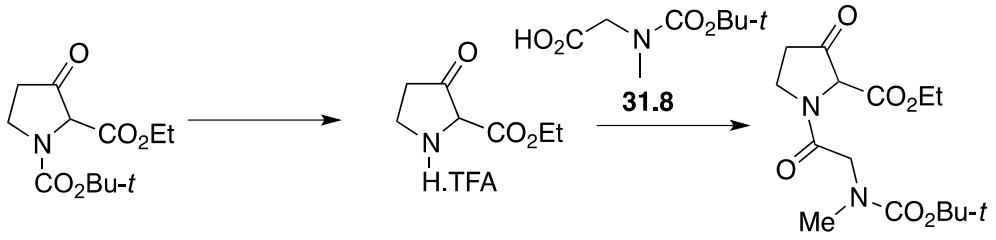
Ethyl (±)-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-3-oxo-pyrrolidine-2-carboxylate (31.3).

31.2 **31.3**

A mixture of PCC (2.3 g, 10.65 mmol) and Celite (2.3 g) was added to a stirred and cooled (0 °C) solution of alcohol **31.2** (1.17 g, 3.55 mmol) in CH_2Cl_2 (80 mL), followed by AcONa (291 mg, 3.55 mmol) and 3Å molecular sieves (1.8 g, 0.5 g/mmol, powdered). Stirring was continued for 30 min, the ice bath was removed and stirring was continued overnight. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. Evaporation of the resulting dark brown filtrate and flash chromatography of the residue over silica gel (3.5 × 20 cm), using 2:1 EtOAc-hexane, gave **31.3** (810 mg, 70 %) as a pale yellow

foam: FTIR ($CDCl_3$, cast) 2978, 2932, 1773, 1741, 1699, 1674 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.26–1.35 (m, 3 H), 1.45 (s, 9 H), 2.60–2.85 (m, 2 H), 2.95 (s, 3 H), 3.90–4.18 (m, 3 H), 4.20–4.38 (m, 3 H), 4.73 (s, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q), 28.3 (q), 35.1 (t) and 37.1 (t), 35.5 (q), 41.6 (t) and 42.2 (t), 50.0 (t), 62.5 (t) and 63.0 (t), 65.2 (d), 80.3 (s), 156.2 (s), 165.3 (s), 168.6 (s), 202.8 (s) and 203.2 (s); exact mass (electrospray) *m*/*z* calcd for $C_{15}H_{24}N_2NaO_6$ $(M + Na)^+$ 351.1527, found 351.1527.

Ethyl (±)-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-3-oxo-pyrrolidine-2-carboxylate (31.3).



28.4b **DP-2** **31.3**

CF_3CO_2H (23 mL) was added to a stirred and cooled (0 °C) solution of **28.4b** (3.8 g, 14.8 mmol) in CH_2Cl_2 (120 mL) and, after 10 min, the cooling bath was removed and stirring was continued for 3 h. The CH_2Cl_2 and excess of CF_3CO_2H were evaporated and the residue **DP-2** was dried under high vacuum for 2 h.

i-Pr_2NEt (10.3 mL, 14.8 mmol) was added to a stirred and cooled (0 °C) solution of **DP-2** and acid **31.8** (2.9 g, 14.8 mmol) in CH_2Cl_2 (80 mL). After 15

min HOBt (3.6 g, 19.2 mmol) and EDCI (3.0 g, 19.2 mmol) were added sequentially. The cold bath was removed and stirring was continued for 16 h. Saturated aqueous $NaHCO_3$ solution (50 mL) was added and the organic phase was washed with dilute hydrochloric acid (1 M, 50 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (3 × 20 cm) , using EtOAc-Hexane, gave **31.2** (2.43 g, 50 %) as a foam: the spectral data are the same as obtained previously.

Ethyl (±)-(4*E*)-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-4-[(dimethylamino)methylidene]-3-oxopyrrolidine-2-carboxylate (34.1).

31.4 **34.1**

$Me_2NCH(OMe)_2$ (1 mL, 6 mmol) was added to a stirred solution of **31.3** (650 mg, 1.98 mmol) in PhMe (30 mL) and the resulting solution was refluxed (80 °C) for 40 min. The solvent was evaporated and the resulting orange solid was washed with Et_2O on a sintered disc to give **34.1** (560 mg, 74 %) as a pale yellow solid: FTIR ($CDCl_3$, cast) 2977, 2930, 1742, 1689, 1592 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.25–1.34 (m, 3 H), 1.42 (s) and 1.46 (s, 9 H), 2.93 (s) and 2.96 (s, 3 H), 3.11 (s) and 3.13 (s, 6 H), 3.40–4.29 (m, 4 H), 4.63–4.71 (m, 1 H),

4.76–4.87 (m, 2 H), 7.36–7.37 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q) and 14.2 (q), 28.1 (t) and 28.4 (t), 35.2 (q) and 35.9 (q), 38.8 (q), 46.5 (q), 47.1 (t) and 47.2 (t), 50.1 (t) and 50.6 (t), 61.9 (t) and 62.4 (t), 65.6 (d) and 65.7 (d), 80.0 (s) and 80.1 (s), 96.3 (s) and 97.2 (s), 148.5 (d) and 149.0 (d), 156.1 (s), 167.7 (s) and 167.8 (s), 168.0 (s) and 168.4 (s), 187.7 (s) and 188.1 (s); exact mass (electrospray) *m*/*z* calcd for $C_{18}H_{29}N_3NaO_6$ $(M + Na)^+$ 406.1949, found 406.1948.

Ethyl (±)-(4*E*)-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-4-[(dimethylamino)methylidene]-2-(methylsulfanyl)-3-oxopyrrolidine-2-carboxylate (34.2).

34.1 **34.2**

DBU (0.36 mL, 2.34 mmol) was added to a stirred and cooled (0 °C) solution of **34.1** (550 mg, 1.43 mmol) and *S*-methyl 4-methylbenzenethiosulphonate[26] (356 mg, 1.75 mmol) in CH_2Cl_2 (30 mL). The cooling bath was removed and stirring was continued overnight. Evaporation of the solvent and flash chromatography of the residue over silica gel (2 × 8 cm), using EtOAc, gave **34.2** (520 mg, 85 %) as a foam: FTIR ($CDCl_3$, cast) 2978,

2927, 1746, 1686, 1593 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.23–1.31 (m, 3 H), 1.42–1.47 (m, 9 H), 2.00–2.10 (m, 3 H), 2.85 (s) and 2.87 (s) and 2.96 (s, 3 H), 3.17 (s, 6 H), 3.63–4.31 (m, 3 H), 4.60–4.88 (m, 3 H), 7.48–7.49 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (q) and 12.5 (q), 14.1 (q), 28.3 (q) and 28.4 (q), 35.3 (q) and 35.5 (q), 38.9 (q) and 39.0 (q), 46.6 (q), 47.7 (t) and 48.1 (t), 50.4 (t), 51.4 (t) and 51.8 (t), 62.6 (s) and 63.5 (s), 79.7 (s) and 80.2 (s), 95.0 (s) and 95.2 (s), 149.1 (d) and 149.7 (d), 156.0 (s) and 156.4 (s), 166.5 (s) and 167.2 (s), 169.6 (s) and 170.0 (s), 187.7 (s) and 187.9 (s); exact mass (electrospray) *m*/*z* calcd for $C_{19}H_{31}N_3NaO_6S$ $(M + Na)^+$ 452.1826, found 452.1826.

(±)-(7*E*)-7-[(Dimethylamino)methylidene]-2-methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4,8-trione (34.3).

34.2 **34.3**

CF_3CO_2H (1.8 mL, 24.2 mmol) was added to a stirred and cooled (0 °C) solution of **34.2** (520 mg, 1.21 mmol) in CH_2Cl_2 (25 mL). The ice bath was removed and stirring was continued for 3 h. The solvent and excess of CF_3CO_2H were evaporated and the residue was dissolved in MeCN (25 mL). The resulting solution was cooled to 0 °C and Et_3N (1.5 mL, 10.4 mmol) was

added dropwise. The ice bath was left in place but not recharged and stirring was continued overnight. The MeCN was evaporated and the residue was diluted with CH_2Cl_2 (30 mL) and washed with saturated aqueous NH_4Cl (20 mL) and brine (20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to give **34.3** (312 mg, 79 %) as a foam which was pure enough for the next step. The material had: FTIR ($CDCl_3$, cast) 2923, 1679, 1593, 1425, 1288 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.38 (s, 3 H), 3.02 (s, 3 H), 3.13 (s, 6 H), 3.78 (d, *J* = 16.5 Hz, 1 H), 4.36 (d, *J* = 16.5 Hz, 1 H), 4.40 (d, *J* = 13 Hz, 1 H), 4.76 (d, *J* = 13 Hz, 1 H), 7.37 (t, *J* = 1 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.7 (q), 34.1 (q), 43.2 (t), 52.6 (t), 62.6 (s), 94.4 (s), 148.7 (d), 164.6 (s), 185.7 (s); exact mass (electrospray) *m*/*z* calcd for $C_{12}H_{17}N_3NaO_3S$ $(M + Na)^+$ 306.0883, found 306.0885.

(±)-(7*E*)-7-(Ethoxymethylidene)-2-methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4-8-trione (34.4).

34.3 **34.4**

TsOH.H_2O (184 mg, 1.07 mmol) was added to a stirred solution of **34.3** (300 mg, 1.07 mmol) in EtOH (30 mL) and the mixture was heated at 70 °C overnight. The solvent was evaporated and the residue was partitioned between

CH_2Cl_2 (20 mL) and water (20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 × 10 cm), using EtOAc, gave **34.4** (280 mg, 93 %) as a white foam: FTIR ($CDCl_3$, cast) 2925, 1732, 1685, 1640, 1434, 1215; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.37 (t, *J* = 7.2 Hz, 3 H), 2.34 (s, 3 H), 3.02 (s, 3 H), 3.81 (d, *J* = 16.8 Hz, 1 H), 4.07 (dd, *J* = 15.6, 2 Hz, 1 H), 4.19 (q, *J* = 7.2 Hz, 2 H), 4.33 (d, *J* = 16.8 Hz, 1 H), 4.53 (dd, *J* = 15.6, 2 Hz, 1 H), 7.51 (t, *J* = 2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.8 (q), 15.4 (q), 34.1 (q), 41.3 (t), 52.5 (t), 62.8 (s), 72.0 (t), 107.3 (s), 156.9 (d), 160.9 (s), 164.2 (s), 186.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_{12}H_{16}N_2NaO_4S$ $(M + Na)^+$ 307.0723, found 307.0729.

(±)-(7*E*,8α,8aα)-7-(Ethoxymethylidene)-8-hydroxy-2-methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4-dione (34.5).

34.4 **34.5**

$NaBH_4$ (40 mg, 1.05 mmol) was added to a stirred and cooled (–15 °C) solution of **34.4** (270 mg, 0.95 mmol) in EtOH (20 mL). The cooling bath was left in place but not recharged and stirring was continued for 2 h. The solvent was evaporated and the residue was partitioned between EtOAc (15 mL) and

saturated aqueous $NaHCO_3$ (15 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 × 8 cm), using 40:1 EtOAc-MeOH, gave **34.5** (180 mg, 68 %) as an oil: FTIR ($CDCl_3$, cast) 3415, 2978, 2924, 1711, 1668, 1439 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.27 (t, *J* = 6.8 Hz, 3 H), 2.15 (s, 3 H), 3.05 (s, 6 H), 3.11 (d, *J* = 3.6 Hz, 1 H), 3.83 (t, *J* = 17.2 Hz, 1 H), 3.90 (q, *J* = 6.8 Hz, 2 H), 4.17–4.19 (m, 2 H), 4.29 (d, *J* = 17.2 Hz, 1 H), 5.00 (s, 1 H), 6.33–6.35 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 12.6 (q), 15.4 (q), 33.5 (q), 43.5 (t), 53.0 (t), 68.7 (t), 72.8 (s), 75.9 (d), 108.3 (s), 142.7 (d), 163.2 (s), 165.9 (s); exact mass (electrospray) *m*/*z* calcd for $C_{12}H_{18}N_2NaO_4S$ $(M + Na)^+$ 309.0879, found 309.0881.

Ethyl (±)-1-(2-{[(*tert*-Butoxy)carbonyl](methyl)amino}acetyl)-2-(methylsulfanyl)-3-oxopyrrolidine-2-carboxylate (31.4).

31.3 **31.4**

Cs_2CO_3 (100 mg, 0.305 mmol) was added to a stirred and cooled (–20 °C) solution of **31.3** (100 mg, 0.305 mmol) and *S*-methyl 4-methylbenzenethiosulphonate[26] (61.6 mg, 0.305 mmol) in MeCN (15 mL) and

stirring at –20 °C was continued for 3 h. The solvent was evaporated and the residue was dissolved in EtOAc (20 mL) and washed with diluted hydrochloric acid (1 M, 15 mL) and brine. The resulting organic extract was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1.5 × 10 cm), using 1:2 EtOAc-hexane, gave **31.4** (60 mg, 52 %, 73 % corrected for recovered starting material) as a colorless oil: FTIR (CDCl3, cast) 2979, 1767, 1744, 1701 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.26 (s, 3 H), 1.45 (s, 9 H), 2.25 (s, 3 H), 2.88 (br, 2 H), 2.94 (s, 3 H), 3.86—4.31 (m, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.5 (q) and 13.6 (q), 13.8 (q) and 14.0 (q), 28.3 (q), 35.0 (t) and 35.2 (t), 35.5 (q), 41.8 (t) and 42.1 (t), 50.1 (t) and 50.8 (t), 62.9 (t) and 64.0 (t), 72.5 (s), 80.1(s) and 80.3 (s), 156.0 (s) and 156.2 (s), 164.9 (s), 167.8 (s) and 168.1 (s), 199.2 (s); exact mass (electrospray) *m*/*z* calcd for $C_{16}H_{26}N_2NaO_6S$ $(M + Na)^+$ 397.1401, found 397.1409.

(±)-2-Methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4,8-trione (32.1).

31.4 **32.1**

CF_3CO_2H (0.75 mL, 9.7 mmol) was added to a stirred and cooled (0 °C) solution of **31.4** (150 mg, 0.4 mmol) in CH_2Cl_2 (15 mL). The ice bath was left in place but not recharged and stirring was continued for 4 h. The solvent and excess of CF_3CO_2H were evaporated and the residue was dissolved in MeCN (10 mL). The resulting solution was cooled to –20 °C and Et_3N (0.3 mL, 1.6 mmol) was added dropwise (about 1 drop per sec). Stirring at –20 °C was continued for 5 h. Evaporation of the solvent and flash chromatography of the residue over silica gel (2 × 8 cm), using EtOAc, gave **32.1** (90 mg, 96 %) as a white foam: FTIR ($CDCl_3$, cast) 2921, 2850, 1766, 1681 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.31 (s, 3 H), 2.60—2.68 (m, 1 H), 2.92 (ddd, *J* = 19, 8.7, 2.5 Hz, 1 H), 3.54 (dt, *J* = 12.1, 8.7 Hz, 1 H), 3.83 (d, *J* = 17.2 Hz, 1 H), 4.15 (dt, *J* = 12.6, 2.6 Hz, 1 H), 4.28 (d, *J* = 17.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.7 (q), 33.3 (t), 34.0 (q), 37.2 (t), 52.5 (t), 60.5 (s), 160.0 (s), 164.1 (s), 195.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_9H_{12}N_2NaO_3S$ $(M + Na)^+$ 251.0461, found 251.0462.

(±)-(8α,8aα)-8-Hydroxy-2-methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4-dione (32.2).

32.1 **32.2**

$NaBH_4$ (47 mg, 1.25 mmol) was added to a stirred and cooled (–78 °C) solution of **32.1** (212 mg, 0.89 mmol) in MeOH (15 mL) and stirring at –78 °C was continued for 1 h. Saturated aqueous $NaHCO_3$ (5 mL) and CH_2Cl_2 (15 mL) were added and stirring was continued at 0 °C for 5 min. The organic solvent was evaporated from the two-phase mixture and the remaining aqueous phase was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 × 8 cm), using 40:1 EtOAc-MeOH, gave **32.2** (180 mg, 85 %) as a white foam: FTIR ($CDCl_3$, cast) 3423, 2956, 2923, 1664 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.19 (s, 3 H), 2.24—2.30 (m, 2 H), 3.04 (s, 3 H), 3.14 (d, *J* = *2*.6 Hz, 1 H), 3.54—3.58 (m, 2 H), 3.79 (d, *J* = 17.2 Hz, 1 H), 4.30 (d, *J* = 17.2 Hz, 1 H), 4.52 (dt, *J* = 9.3 Hz, 2.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.9 (q), 26.5 (t), 33.4 (q), 40.6 (t), 53.2 (t), 71.2 (s), 76.3 (d), 163.4 (s), 166.2 (s); exact mass (electrospray) *m*/*z* calcd for $C_9H_{14}N_2NaO_3S$ $(M + Na)^+$ 253.0617, found 253.0619.

(±)-(8α,8aβ)-2-Methyl-8a-(methylsulfanyl)-1,4-dioxooctahydro-pyrrolo[1,2-*a*]piperazin-8-yl 4-nitrobenzoate (32.3).

32.2 **32.3**

Diisopropyl azodicarboxylate (0.12 mL, 0.768 mmol) was added to a stirred and cooled (–40 °C) solution of **32.2** (70 mg, 0.307 mmol), 4-nitrobenzoic acid (120 mg, 0.676 mmol) and Ph_3P (200 mg, 0.768 mmol) in THF (3 mL). The cooling bath was removed and stirring was continued for 36 h. Evaporation of the solvent and flash chromatography of the residue over silica gel (1.5 × 15 cm), using 1:2 EtOAc-hexane, gave **32.3** (100 mg, 87 %) as a white solid: FTIR ($CDCl_3$, cast) 3111, 3077, 2962, 1731, 1676, 1527, 1433 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.23 (ddd, *J* = 15.0, 8.0, 1.5 Hz, 1 H), 2.28 (s, 3 H), 2.88 (ddt, *J* = 14.5, 10.0, 4.5 Hz, 1 H), 3.00 (s, 3 H), 3.88 (d, *J* = 17.0 Hz, 1 H), 3.78—3.90 (m, 2 H), 4.33 (d, *J* = 17 Hz, 1 H), 5.93 (d, *J* = 4.0 Hz, 1 H), 8.04 (dt, *J* = 9.0 Hz, 2.0 Hz, 2 H), 8.26 (dt, *J* = 9.0 Hz, 2.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q), 27.2 (t), 33.8 (q), 43.8 (t), 53.0 (t), 72.4 (s), 76.8 (d), 123.6 (d), 130.8 (d), 135.1 (s), 150.7 (s), 162.0 (s), 163.4 (s), 163.7 (s); exact mass (electrospray) *m*/*z* calcd for $C_{16}H_{17}N_3NaO_6S$ $(M + Na)^+$ 402.0730, found 402.0727.

(±)-(8α,8aβ)-8-Hydroxy-2-methyl-8a-(methylsulfanyl)octahydropyrrolo[1,2-*a*]piperazine-1,4-dione (32.4).

32.3 **32.4**

Method 1:

A solution of $LiOH \cdot H_2O$ (13 mg, 0.317 mmol) in water (2 mL) was added to a stirred and cooled (0 °C) solution of **32.3** (60 mg, 0.158 mmol) in THF (6 mL) and stirring at 0 °C was continued for 20 min. Water (10 mL) and EtOAc (10 mL) were added and aqueous phase was extracted with EtOAc (5 × 10 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (1 × 8 cm), using EtOAc, gave **32.4** (39 mg, 65 %) as a white solid: FTIR ($CDCl_3$, cast) 3355, 2954, 2850, 1657 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.06 (dd, *J* = 13.8 Hz, 7.4 Hz, 1 H), 2.18 (s, 3 H), 2.49–2.56 (m, 1 H), 3.05 (s, 3 H), 3.19 (s, 1 H), 3.70 (t, *J* = 10.2 Hz, 1 H), 3.77 (d, *J* = 16.8 Hz, 1 H), 3.85 (dd, *J* = 15.8, 8.2 Hz, 1 H), 4.35 (d, *J* = 16.8 Hz, 1 H), 4.62 (d, *J* = 4.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.0 (q), 27.5 (t), 33.7 (q), 44.4 (t), 53.2 (t), 74.0 (s), 75.6 (d), 163.9 (s), 164.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_9H_{14}N_2NaO_3S$ $(M + Na)^+$ 253.0617, found 253.0616.

Method 2:

NaN_3 (82 mg, 1.27 mmol) was added to a stirred solution of **32.3** (160 mg, 0.422 mmol) in $MeOH/CH_2Cl_2$ (15 mL, 2:1) and the resulting mixture was heated to 40 °C and stirred for 24 h. Solvent was evaporated and flash chromatography of the residue over silica gel (2 × 8 cm), using EtOAc, gave **32.4** (72 mg, 74 %) as a white solid.

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