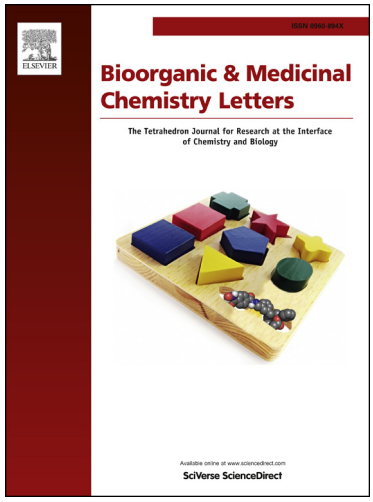

Discovery of novel P2 substituted 4-biaryl proline inhibitors of hepatitis C virus NS3 serine protease

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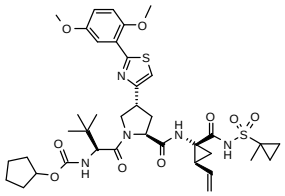

IC_{50} = 1.1 nM
EC_{50} = 9 nM

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Discovery of novel P2 substituted 4-biaryl proline inhibitors of hepatitis C virus NS3 serine protease

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ABSTRACT

Inhibitors of hepatitis C virus NS3 serine protease often incorporate a large P2 moiety to interact with the surface of the enzyme while shielding part of the catalytic triad. This feature is important in many inhibitors in order to have the necessary potency needed for efficacy. In this paper we explore some new P2 motifs to further exploit this region of the enzyme. In a continuing effort to replace the often found 4-hydroxyproline P2 core found in the majority of inhibitors for this target, various directly attached aryl derivatives were evaluated. Of these, the 2,4-disubstituted thiazole core proved to be the most interesting. SAR around this motif has lead to compounds with K_i's in the high picomolar range and provided cellular potencies in the single digit nM range.

Hepatitis C virus (HCV) infection has been estimated to infect approximately 2% of the world's population[1] and is the leading cause of chronic inflammation of the liver leading to liver cirrhosis, hepatocellular carcinoma, and liver failure in humans. Until recently, treatment consisted of a combination of pegylated interferon agents with ribavirin, a broad range nucleoside antiviral. This therapy is known to be suboptimal for a large number of patients and new treatments were needed in order to reach more patients who presently respond poorly to the current regiment.[2] The first direct acting antivirals agents (DAA) boceprevir (Victrelis) and telaprevir (Incivek/Incivo) were recently introduced as an add on to the current therapy, considerably improving the response rate.[3] A number of other agents are now in late-stage clinical development.[4]

The first proof-of-concept study for a NS3/4A protease inhibitor originated from our laboratories with BILN 2061 (ciluprevir), a macrocyclic inhibitor containing a C-terminal carboxylic acid.[5] While development of ciluprevir was discontinued, linear analogs also based on peptide substrates were pursued leading ultimately to faldaprevir (BI 201335),[6] which is completing phase III clinical trials. A number of other macrocyclic and linear analogs of NS3 protease inhibitors have appeared in the literature, many of which contain acid bioisosteres or active site covalent reversible binding groups.[7,8,9,10] In particular, introduction of an acyl-sulfonamide bioisostere[11] at the C-terminal end of these substrate-based inhibitors was found to improve potency dramatically allowing for truncations at other sites of the inhibitor.

We have successfully shown that a substituted P2 hydroxyproline moiety as found in both ciluprevir and faldaprevir **1** is a valuable fragment in the development of protease inhibitors. The nature of this group is thought to shield

part of the catalytic triad from water, namely Asp81 and His57.[12] While a number of protease inhibitors have incorporated smaller non-aromatic P2 derived proline derivatives such as boceprevir, telaprevir and narlaprevir[13], the majority of the current clinical candidates still contain variations of the hydroxyproline fragment originally found in ciluprevir[5]. Interestingly, the smaller non-aromatic P2 containing inhibitors rely on the incorporation of covalent reversible P1/P1' binding groups for potency while the larger hydroxyproline derivatives rely on ionic interactions in the P1/P1'region, often incorporating an acyl-sulfonamide group. In our continuing efforts to diversify and exploit the P2 region of the protease, several novel P2 arrangements have been reported by us including directly attached 4-triazole substituted proline analogs **2**[14] and more recently 4-phenyl substituted prolines derivatives **3** (Scheme 1)[15]. It was shown in these later two examples that a biaryl group attached to the 4-position of the P2 proline moiety was necessary to obtain the desired level of potencies for these series. In this paper, we continue to explore these motifs by examining alternatives to the triazole core found in compound **2** in order to generate novel P2 proline fragments and to discover new ways of interacting with the S2 region of the enzyme.

As a starting point, we first replaced the central triazole ring found in compound **2** with other heterocyclic ring systems in Scheme 2 where the IC_{50} values are from a genotype 1 assay and the EC_{50} values from a replicon assay[16]. Replacement of the triazole ring by either an N-linked phenyl imidazole **4** or phenyl pyrazole ring **5** did not lead to any improvement in the overall profile of this series. The imidazole ring in particular was least tolerated due probably to the more polar nature of this group. Substituting the central ring for either the 2- or the 4-attached thiazolyl phenyl rings (compounds **6** and **7**) produced compounds with good potency and generally improved HLM stability and Caco-2 values over the other analogs. Of

the two isomeric thiazolyl derivatives, compound **7** exhibited the best overall profile and therefore was selected for further studies.

Beginning with the phenyl derivative **7**, several heterocyclic replacements of the terminal phenyl group were prepared and exemplified by the pyridine analog **8** in Table 1. None of these type of ring replacements were beneficial to cellular potency, likely a result of the lipophilic nature of the pocket. Retaining the phenyl group as in compound **7**, mono-substitution on the ring was explored by first walking a methoxy group around the ring as shown with compounds **9**, **10**, and **11**. Interestingly, while the para and meta substitutions were tolerated (compounds **9** and **10**), the ortho substituted analog **11** resulted in an important ortho substituent effect. This substitution resulted in nearly a 4-fold improvement in intrinsic potency and a 5-fold improvement in cellular potency over compound **7**, delivering our first sub-nM potency compound in the series. With the discovery of this ortho methoxy group effect, a number of other small ortho ether substituents were screened including the ethoxy group (compound **12**), however these analogs were all equipotent to **11**. Other ortho groups evaluated included among others the Me and F analogs (compounds **13**, **14**) which were detrimental to potency. With the finding of this ortho methoxy group effect on potency, we revisited the regioisomeric 4-phenylthiazol-2-yl analog (not shown) of compound **11** and found this to be more than 3-fold less potent, consistent with our original finding.

Having established this novel P2 moiety, a molecular model based on in-house X-ray structures was created with compound **11** in the presence of the NS3 protease as shown in Figure 1, Panel A. The thiazole moiety was shown to adopt a pseudo axial conformation on the P2 proline ring which allows for the phenyl group to nestle against the P2 binding pocket, similar to that described previously

with Arg155 being desolvated and forming a salt-bridge with Asp168[17]. The biaryl groups are modelled nearly coplanar (~12^0) allowing the two rings to conform to a slight ridge formed between residues Asp81 and Arg155. The important ortho methoxy group is oriented with its oxygen atom largely solvated and its methyl group filling a small pocket adjacent to the backbone carbonyl oxygen of Asp79. It is interesting to note that this same position was central to a key halogen bond in the binding of faldaprevir[17]. It is possible that the favourable interactions of the methyl group within this pocket explain the superiority of compound **11** over compounds **13** and **14**. This model also suggested that substitution at the 3-position is largely blocked by Val78, but further favorable surface interactions with the P2 pocket may be achieved with substitutions at the 5-position on the phenyl ring.

Armed with this information, we next explored the possibility of incorporating a second substituent around the phenyl ring as shown in Table 2. Systematic substitution at each of the available positions on the phenyl ring with a second methoxy group was first explored. While substitution at the 3- and 4-positions as in compounds **15** and **16** were somewhat tolerated, substitution at the 6-position was the least tolerated likely due to a reinforcement of a non-planar orientation and the seemingly tight fit in the pocket resulting in the significant loss in potency observed for compound **17**. As suggested by the modeling study, the best placement of the second methoxy group at the 5-position provided the 2,5-disubstituted analog **18** which gave an improvement in cellular potency by 2-fold over compound **11**. Next, the 5-methoxy group was replaced by other substituents as found in the Me and F analogs (compounds **19** and **20**) but none of these analogs reached the potency of compound **18**. Since compound **18** was expected to be near the wall of the IC_{50} assay, the K_i value was determined for this particular compound and found to be in the range of 230 ρM. In order to improve potency

further, introduction of a methyl -substituted cyclopropane on the terminal acyl sulfonamide generated compound **21** with an IC_{50} = 1.1 nM and EC_{50} = 9 nM which represented our most potent compound in this series. Some ADME properties were determined for these two analogs (Scheme 3). Both compounds had modest stability against human liver microsomes (**18**: HLM = 69 min, **21**: HLM = 57 min) but good cell permeability as measured by the Caco-2 model (**18**: 7 x 10^{-6} cm/sec, **21**: 12 x 10^{-6} cm/sec).

As a further exercise, analogs from three distinct series were superimposed in the model to visualize the subtle differences in the P2 group orientations (Figure 1, Panel B). As can be seen from this figure, the 2-phenyl-4-thiazolyl proline derivative **11** samples different space relative to the biphenyl type analogs highlighted by fragment **3** or the more extensive P2 hydroxyproline analog found in faldaprevir. This comparison visualizes compound **11** being shifted more towards the P1 residue relative to the biphenyl analog **3** and occupied a much smaller region than the hydroxyproline derivative **1**. Subtle differences in the orientations and size of these P2 derivatives could have important implications for the resistance profiles of these different series. From preliminary data, we found compound **18** shifted 8 and 10-fold for mutations A156T and D168V respectively, somewhat less than analogous hydroxyproline derivatives (data not shown).

The synthesis of the 2-aryl substituted 4-thiazole analogs have been exemplified by the preparation of compound **7** as outlined in Scheme 4. Starting from brosylate **22** which has previously been described[5], potassium cyanide displacement of the brosylate group under thermal conditions gave nitrile **23**. The nitrile group was hydrolyzed under acidic conditions using MeOH-HCl followed by the selective hydrolysis of the methyl ester intermediate with 1N NaOH to afford the mono acid **24**. Using a 3-step protocol, acid **24** was first activated with isobutyl chloroformate

at 0°C followed by treatment with a solution of diazomethane. Following a simple work-up in EtOAc and saturated aqueous $NaHCO_3$, the crude material was purified by SiO_2 chromatography to give the pure diazoketone intermediate. This intermediate was next treated dropwise at 0°C with 48% aq. HBr over several minutes before being poured into EtOAc and quenched with saturated aqueous $NaHCO_3$ to supply the α-bromoketone intermediate **25** in nearly quantitative yield. The α-bromoketone was then condensed with thiobenzamide at 65°C followed by the hydrolysis of the terminal ester with 1N NaOH at rt (16h) to give the corresponding acid **26**. Other analogs were prepared from this key α-bromoketone intermediate by substituting the appropriate aryl thioamide derivatives. Activation of **26** with isobutyl chloroformate generated the azalactone intermediate **27** which was subsequently opened with cyclopropane sulfonamide under LiHMDS conditions. The regioisomeric 4-phenyl-2-thiazolyl analog **6** was prepared by conversion of the cyano intermediate **23** to the corresponding thioamide in a sealed tube with H_2S and NEt_3/dioxane. The corresponding thioamide was then treated with the appropriate α-bromoketone to generate the desired thiazole ring system, followed by the same sequence as outlined in Scheme 4. The N-linked analogs (compounds **4** and **5**) were prepared by a simple S_N2 displacement of the brosylate intermediate with the desired functionalized heterocycle.

In summary, we have reported on our continuing effort to diversify and provide novel P2 fragments to inhibit the HCV NS3 serine protease. This study provided a new series of potent 2-phenyl-4-thiazolyl proline analogs which incorporates an acyl-sulphonamide P1' residue required for the level of potency needed for successful clinical compounds of this target. From modeling studies this new P2 moiety occupies the S2 pocket in a slightly different manner than the hydroxyproline or the 4-biarylproline analogs previously described by our group.

The two beneficial methoxy groups affixed to the phenyl ring both sample different areas of the S2 region of the enzyme. The ortho methoxy group on the terminal phenyl ring conforms to a slight ridge between Asp81 and Arg155 whereas the 5-methoxy group occupies open space in the opposite direction. These two interactions improved potency significantly for this series and provided compounds **18** with an IC_{50} value of 0.9 nM and a K_i value in the high picomolar range. Our most potent compound in the replicon assay was compound **21** with an IC_{50} = 1.1 nM and EC_{50} = 9 nM. Both compounds exhibited good initial profiles for both the stability and permeability data which would hopefully translate into favourable parameters for PK studies. The syntheses of these new 4-thiazolyl proline analogs were readily accomplished by way of a common α-bromoketone intermediate developed in our laboratory.

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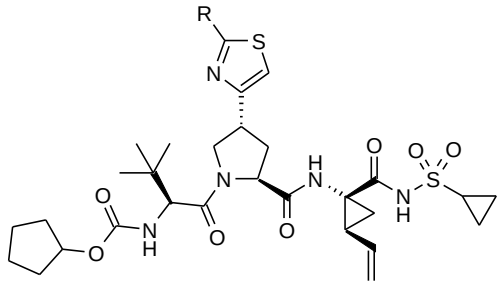
Table 1. Modification of Terminal Aryl Group



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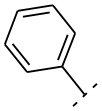
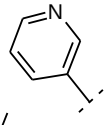
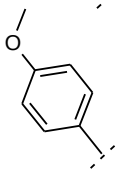
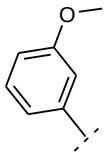
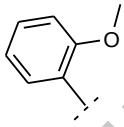
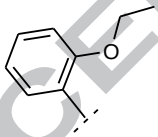
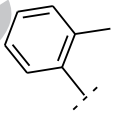
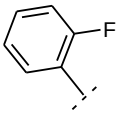
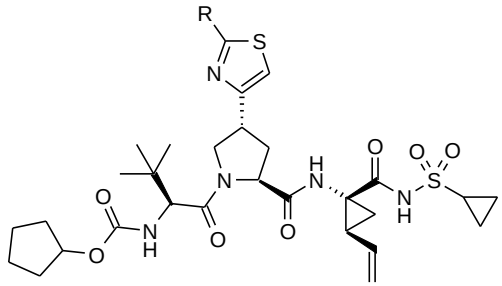
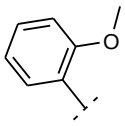
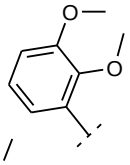
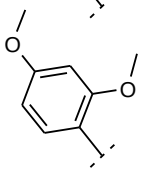
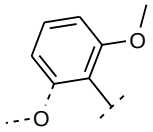
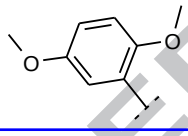
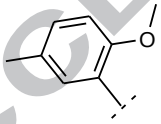
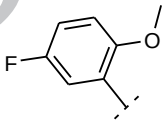
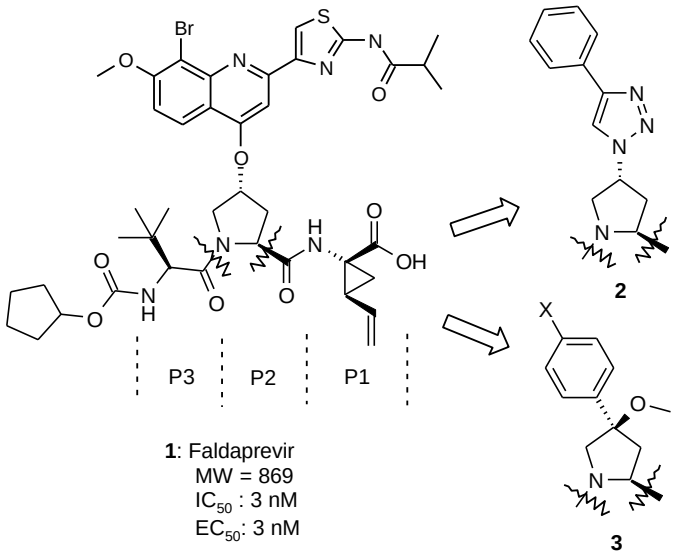
7		2.7	175
8		12	915
9		5.2	530
10		3.4	140
11		0.74	35
12		0.78	37
13		6.0	800
14		1.8	235

Table 2. Disubstitution Study of Aryl Group

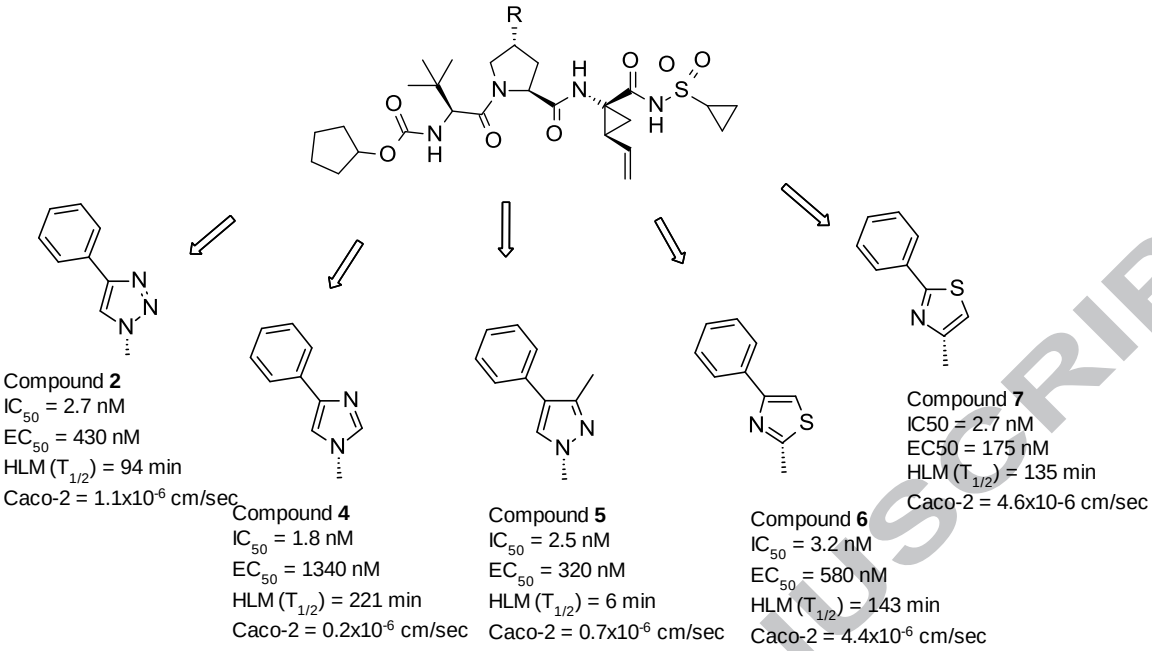


Compound	R group	IC_{50} (nM)	EC_{50} (nM)
11		0.74	35
15		4.1	240
16		2.2	89
17		17	2200
18		0.9	16
19		1.7	27
20		1.1	28

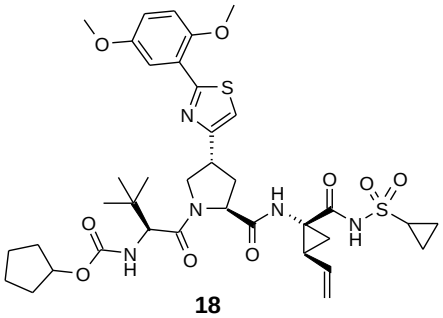


1: Faldaprevir
MW = 869
IC_{50} : 3 nM
EC_{50}: 3 nM

Scheme 1. Modification of the central P2 proline fragment



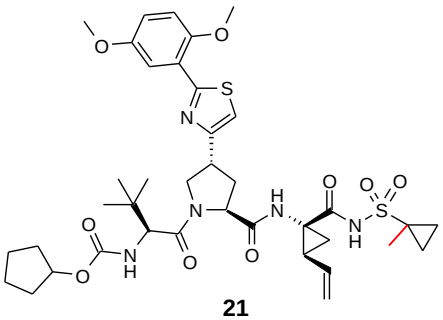
Scheme 2. Effect on Modification of the Central Heterocyclic Core (IC_{50}'s are gt1)



18

IC_{50} = 0.9 nM (K_i = 230 pM)
EC_{50} = 16 nM

Stability: HLM = 69 min
Caco-2 permeability = 7 x 10^{-6} cm/sec

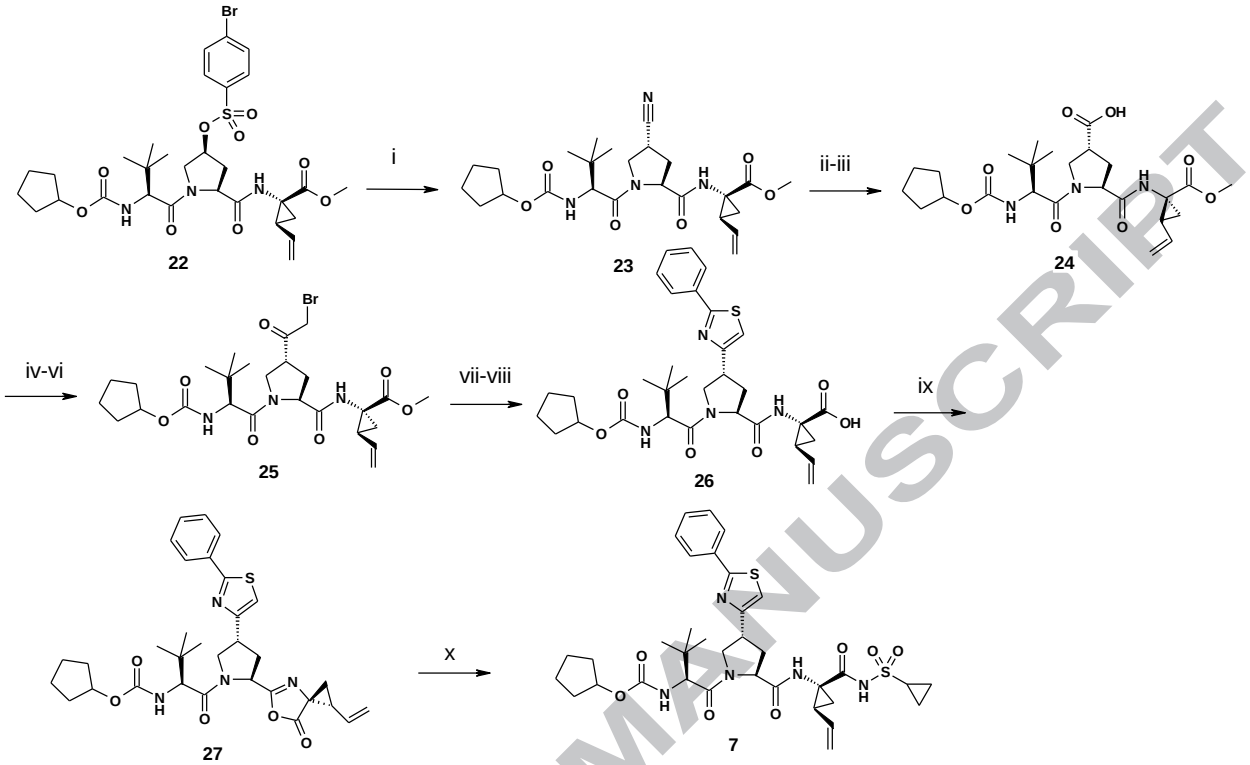


21

IC_{50} = 1.1 nM
EC_{50} = 9 nM

Stability: HLM = 57 min
Caco-2 permeability = 12 x 10^{-6} cm/sec

Scheme 3. Profile of compounds **18** and **21**



Scheme 4. i) KCN in DMSO, 55^{o}C, 24h, 97% (ii) MeOH-HCl, 16 h, 100% (iii) THF, MeOH, H_2O, 1N NaOH, 1.5h, 96% (iv) THF, NEt_3, IBCF, 0^{o}C, 1.5h (v) Diazomethane in ether, 0^{o}C, 1h, 49% over 2-steps (vi) THF 48% aq. HBr, 0^{o}C, 30 min, 99% (vii) THF, thiobenzamide, 65^{o}C, 1h (viii) MeOH, H_2O, 1N NaOH, rt, 16h, 100% over 2-steps (ix) DCM, IBCF, NEt_3, 1h, 100% (x) cyclopropane sulfonamide, THF, at - 15^{o}C, 1M LHMDS, warm to rt, 16h, 51%.