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Cite this: DOI: 10.1039/c0xx00000x

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COMMUNICATION

Scalable asymmetric synthesis of a key fragment of Bcl-2 / Bcl-$_{XL}$ inhibitors†

Sylvain Laclef,[a‡] Catherine Taillier,[a‡] Christine Penloup,[b] Aurélie Viger,[b] Jean-François Brière,[a] Christophe Hardouin*[b] and Vincent Levacher*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

The asymmetric synthesis of a 1,3-diamine building block for the elaboration of Bcl-2 and Bcl-$_{XL}$ protein inhibitors is described through two key steps (1) a highly diastereoselective aza-Reformatsky reaction and (2) a chemoselective amination upon Mitsunobu conditions. This synthetic sequence was also demonstrated to be successfully amenable to a large-scale synthesis.

Defects in the apoptotic processes[1] is playing an important role along tumour initiation, progression and chemoresistance.[2] Among the apoptosis regulator Bcl-2 family (B-cell lymphoma 2), the anti-apoptotic Bcl-2 and Bcl-$_{XL}$ proteins were found to be overexpressed in many cancers.[3-5] Within a complex orchestration to regulate cell fate, the anti-apoptotic Bcl-2 and Bcl-$_{XL}$ proteins and others inhibit pro-apoptotic proteins such as BAK and BAX. Importantly, these interactions can be antagonised by BH3-only proteins (BAD, BIM and NOXA), possesses a single BH domain displaying a large hydrophobic groove with the same fold. Consequently, the development of small molecules BH3 mimetics as inhibitors of anti-apoptotic Bcl-2 and Bcl-$_{XL}$ proteins are attractive targets for novel anticancer therapy.[6-9]

A fragment-based drug design[10] approach led to the discovery of several[11] potent Bcl-2 and Bcl-$_{XL}$ inhibitors such as **1** in Abbott Laboratories (ABT-737, Figure 1).[12] Analogues based on similar scaffold were recently developed.[13] In that field of research (Figure 1), Servier Laboratories developed conformationally restricted isosters **2** which displayed submicromolar activity. The tricyclic architecture aimed at addressing both the solubility issues and at modulating the interactions with the hydrophobic groove of the proteins. Extensive structure-activity relationship studies revealed the essential importance of common diamine fragment such as **3**,[14] containing a 1,3-diamine moiety flanked by a phenylthioethyl arm, for securing both biodisponibility and potent inhibition of Bcl anti-apoptotic proteins. These outcomes highlighted that the *R* isomer displayed better bioactivity than the opposite enantiomer.

As far as the construction of diamine fragment **3** is concerned, only one chiral pool based synthesis was reported using L-aspartic acid precursor.[15] This method allowed the synthesis of compound **3** in 8 steps and 30% overall yield.

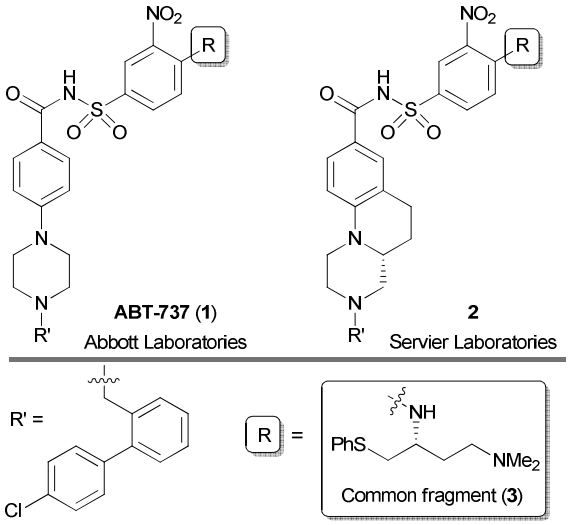
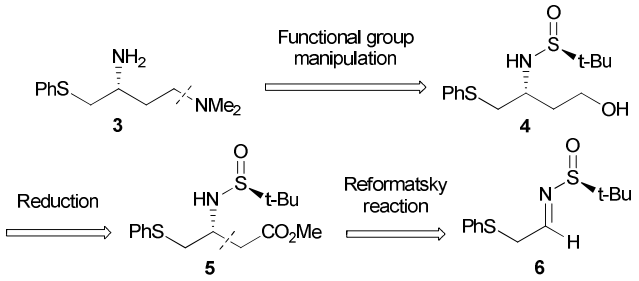


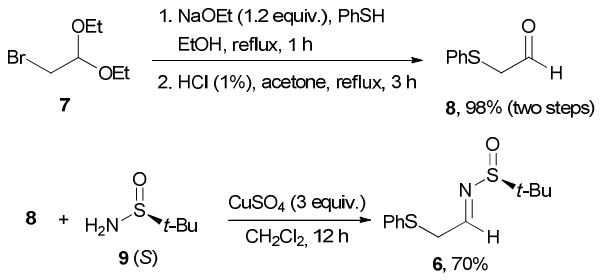
Fig. 1 Structures of Bcl-2 / Bcl-$_{XL}$ inhibitors.

In this context, we endeavoured to develop a reliable access towards diamine **3** through an alternative asymmetric synthesis approach. The aim is eventually to get a flexible larger-scale synthetic sequence en route to provide significant amounts of Bcl-2 protein inhibitor from key building block **3**. The retrosynthetic approach was based on both diastereoselective aza-Reformatsky (**6** to **5**) and chemoselective amination key reactions (**4** to **3**, Scheme 1). First of all, the chiral Ellman's *N-tert*-butanesulfinamide, readily available on a large scale as both enantiomers, was selected as a versatile chiral auxiliary for the asymmetric synthesis of amine **5**.[16] However, despite previous examples reporting the use of chiral Ellman's sulfinimines in Reformatsky reaction,[17] the influence of the thioether functionality of **6** on both reactivity and diastereoselectivity remains an open issue. Then, we sought to capitalize on the *N*-sulfinyl protecting group of **5** in order to perform further functional group manipulation like the key chemoselective amination step on **4**. We are pleased to report herein our efforts towards the development of a scalable diastereoselective synthesis of chiral scaffold **3**, a useful building and potentially versatile block in medicinal chemistry.



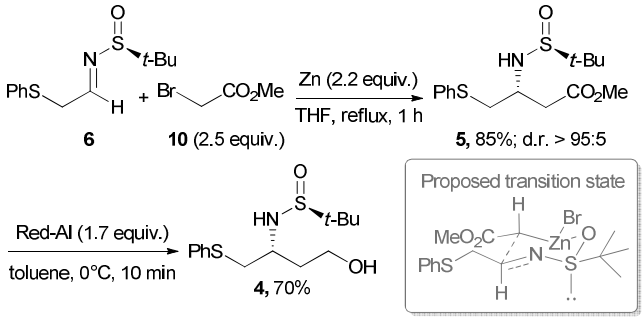
Scheme 1 Asymmetric synthesis approach of diamine fragment.

According to literature procedures, bromoacetaldehyde acetal **7** was converted to the aldehyde precursor **8** in two steps (Scheme 2).[18] The transformation of the rather unstable aldehyde **8** into the corresponding enantiomerically pure *N*-(*tert*-butylsulfinyl)imine **6** was successfully carried out with copper(II) sulfate as dehydrating agent in 70% yield.[16] These conditions were superior to the standard use of $Ti(OEt)_4$ which gave **6** in only 52% yield. It should be noticed that other chiral auxiliaries such as (*R*)-1-phenylethylamine or (*R*)-2-methoxy-1-phenylethylamine failed to give the corresponding imines, highlighting the robustness of the Ellman's sulfinamides approach.



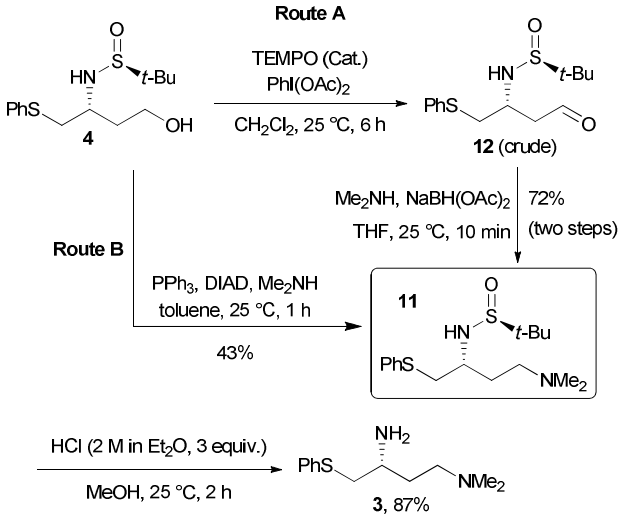
Scheme 2 Synthesis of sulfinimine intermediate **6**.

Then, the sulfinimine **6** was treated with an excess of the Reformatsky reagent derived from the corresponding bromo acetate **10** (2.2 equiv.) under Barbier's conditions (Scheme 3).[19] Pleasingly, the desired product **5** was obtained in 85% yield with high diastereoisomeric ratio (d.r. > 95:5). Actually, changing the reaction temperature from 50 to 0 °C had negligible impact on d.r. although providing lower yields in some cases. By means of Red-Al, the methyl ester **5** was easily reduced to furnish the desired (*R*)-alcohol **4** in 70% yield. Based on Ellman's model, already applied to the Reformatsky reagent originated from **10**, we proposed the following explanation to account for the diastereoinduction outcome.[16,17c] Considering that the Reformatsky reagent derived from methyl bromoacetate **10** exists as a monomeric C-metallated species in polar solvents,[20] a regular Zimmerman-Traxler transition state involving a six-membered intermediate with zinc metal coordinated to the sulfinyl oxygen is proposed. Then, the nucleophilic attack of the Reformatsky reagent to the *Re* face of imine is taking place (Scheme 3). The high diastereoselectivity obtained demonstrates that the putative coordination between sulfinimine and zinc is not disturbed by other complexing function such as the thioether moiety.



Scheme 3 The key Reformatsky reaction.

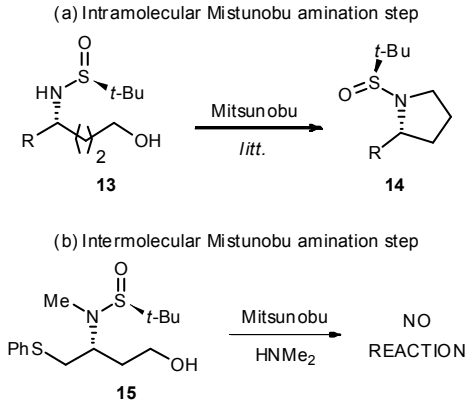
Two different pathways were next considered to transform the primary alcohol **4** into the tertiary amine **11** (Scheme 4). First, following a two-step sequence, the alcohol **4** was converted quantitatively to aldehyde **12** using the mild 2,2,6,6-tetramethyl-1-piperidinyloxyl and [bis(acetoxy)-iodo)]benzene (TEMPO-BAIB) oxidative system (route A, Scheme 4).[21] Then, the crude aldehyde **12** underwent a reductive amination sequence in the presence of $NaBH(OAc)_3$ to give amine **11** in 72% overall yield. Unfortunately, we met important reproducibility issues due to the instability of aldehyde **12** when attempting to scale-up the reaction. To overcome these difficulties, an alternative approach (route B) based on a one-step Mitsunobu reaction with dimethylamine was studied.[22] This strategy led to the formation of product **11** with a respectable yield of 43 % and, more importantly, with a robust scalable protocol (*vide infra*). Finally, the deprotection of *N*-sulfinyl functional group of **11** was achieved upon regular acidic conditions affording the target diamine molecule **3** in 87 % yield. The *R*-absolute configuration was assigned at that stage by comparison with the optical rotation previously reported.[10]



Scheme 4 Completion of the synthesis of diamine fragment **3**.

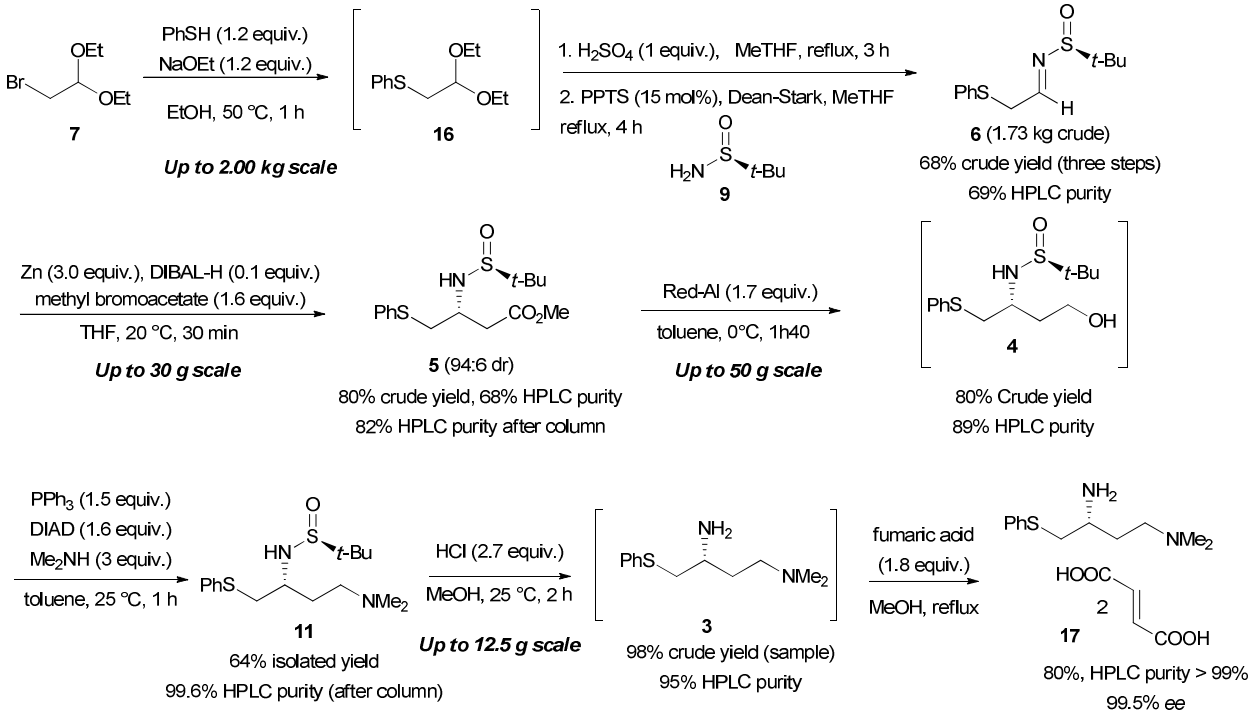
It is worth pointing out that the outcome of the Mitsunobu reaction (Route B, Scheme 4) is surprising considering the low acidity of both primary alcohol **4** and dimethylamine starting materials, especially in the presence of the NHSO*t*-Bu moiety. Indeed, it was reported in the literature that *tert*-butylsulfinamines **13** can react intramolecularly with a proximate alcohol to form a

five-membered pyrrolidine ring **14** (Scheme 5a).[23] In our case, the formation of a four membered azetidine ring should be more energetically demanding.[24] Moreover, we could demonstrate (see supporting information) that the *N*-methylated precursor **15** did not react upon the Mitsunobu conditions with dimethylamine (Scheme 5b). Therefore, we assume that the NH bond favours the formation of the phosphonium intermediate allowing the subsequent nucleophilic substitution to take place, even with dimethylamine having a high pK_a value. On the other hand, the formation of a phosphorane intermediate could not be ruled out.[25]



Scheme 5 Inter- versus intra-molecular amination upon Mitsunobu conditions.

Then, we embarked along a larger scale synthesis of diamine target **3** by optimizing our validated sequence with special attention being paid to minimize the number of purification procedures initially required at each reaction step (Scheme 6). Subsequent to the easy formation of sulphide **16** on a 2 kilogram scale, the acetal deprotection into aldehyde **8** was performed with H_2SO_4 in order to prevent the use of corrosive HCl. Keeping the green solvent MeTHF as reaction media, the formation of imine **6** was conveniently carried out by means of a Dean-Stark distillation in the presence of the soft PPTS acid. This allowed the formation of sulfinimide **6** in 68% crude yield (see supporting information) on a kilogram scale through three telescoped steps (**7** to **6**). Though the purity of the product was estimated to 69% by means of HPLC analysis, this quality turned out to be sufficient for the subsequent steps. Disappointingly, a solvent screening revealed that the next Reformatsky reaction led to partial conversions in MeTHF solvent. Further optimisation and switching to THF demonstrated that imine **6** was completely transformed into amine **5** with a high diastereoisomeric ratio of 94:6, after a soft citric acid work-up to preserve the chiral auxiliary. Worthy of note, according to a literature procedure,[17d] the activation of zinc metal by DIBAL-H was preferred so that the initially uncontrollable exothermicity is avoided during the Reformatsky's reagent formation. A column chromatography on silica gel improved the purity of product **5**, from 68% to 82% as estimated by HPLC analysis, which was found to be sufficient for the next step. The reduction of the ester group by Red-Al (**5** to **4**) and the subsequent Mitsunobu reaction were next successfully telescoped in the same solvent to furnish the crude amine **11.** A silicagel column chromatography was required to remove the large amount of triphenylphosphine side product, which allowed the isolation of amine **11** in a good 64% yield and more than 99% purity measured by HPLC. Moreover, the isolation of the pure major diastereoisomer of **11** was secured at this stage. The chiral auxiliary was removed by HCl in methanol, and the corresponding diamine **3** was obtained in toluene solution after neutralization. Then, the final product **17** was conveniently isolated as a solid fumarate salt which furnished a pure material in 99.5% *ee*. Worthy of note, all attempts to perform the one-step deprotection of sulfinamine **11** by fumaric acid was unsuccessful.



Scheme 6 Scale-up synthesis of diamine building block **3** fumarate salt.

Conclusions

A novel asymmetric synthesis of an enantiopure 1,3-diamine **3**, a key fragment of potent Bcl-2/Bcl-$_{XL}$ protein inhibitor, was accomplished in seven linear steps. The two key steps involve both a highly diastereoselective aza-Reformatsky reaction on a chiral sulfinimine and a chemoselective Mitsunobu reaction allowing the introduction of the dimethylamine moiety. This laboratory synthesis of diamine **3** was demonstrated to be amenable to a larger scale process performed up to kilogram scale for some steps and required only two purifications by column chromatography. Both enantiomers of diamine **3** were shown to be available as useful building blocks of bioactive material.

Acknowledgements

This work has been partially supported by INSA Rouen, Rouen University, CNRS, EFRD, Labex SynOrg (ANR-11-LABX-0029) and Région Haute-Normandie (CRUNCH network). We warmly thank J.-P. Lecouvé, the pilot plant team and the analytical team for their valuable support.

Notes and references

[a] *Normandie UNIV, COBRA, UMR 6014 et FR3038; Univ Rouen; INSA Rouen; CNRS, IRCOF, 1 rue Tesnière, 76821 Mont Saint Aignan Cedex, France. E-mail: vincent.levacher@insa-rouen.fr*

[b] *Oril Industrie, 13 rue Auguste Desgenétais, 76210 Bolbec, France. E-mail: christophe.hardouin@fr.netgrs.com*

† Electronic Supplementary Information (ESI) available: For procedures and compound characterisation. See DOI: 10.1039/b000000x/

‡ These two researchers equally contributed to this project.

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22 The more classical two-step approach, namely the *N*-alkylation of dimethylamine by means of mesylated or tosylated alcohol **4** was also abandoned because of eratic outcomes obtained during the

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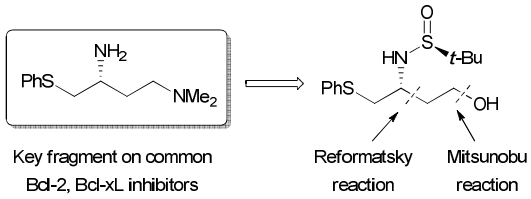

We described a novel asymmetric synthesis, applicable to a large-scale, of a chiral diamine useful as a common fragment of numerous Bcl-2 and Bcl-x_L inhibitors.