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Total Synthesis of Daphlongeranine B

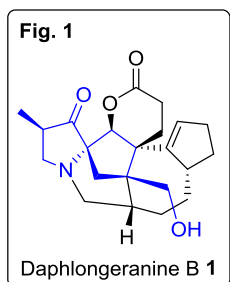
“DAPHNISYN”

TOTAL SYNTHESIS OF DAPHLONGERANINE B

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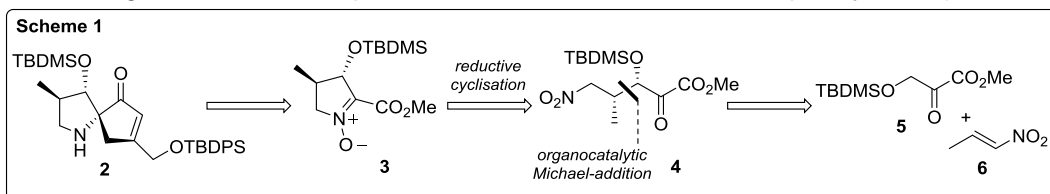
1. WORK PROGRESS AND ACHIEVEMENTS DURING THE PERIOD

Our proposal is aimed at the first total synthesis of *Daphniphyllum* alkaloid Daphlongeranine B (Figure 1). To date, more than 200 members of this family of natural products have been isolated and structurally characterised; some of them have been shown to possess interesting biological activity, such as cytotoxic- or antioxidative properties. However, not least due to their limited availability from natural sources, the biological functions and pharmacological properties of *Daphniphyllum* alkaloids have been only poorly studied until now.¹ All the more surprising, the overall number of completed total syntheses until now is relatively little.² Our target molecule, Daphlongeranine B (**1**) (Figure 1) was isolated from the fruits of *Daphniphyllum longeracemosum* in 2007³ and was shown to exhibit in vitro platelet aggregation. Its unprecedented hexacyclic core with seven stereogenic centres, three of which are fully substituted, presents a formidable and as yet *unmet synthetic challenge*. In our endeavours to elaborate a synthetic route towards the architecturally complex skeleton of our target, we identified spirocyclic pyrrolidone **2** (Scheme 1) as a key building block with two stereogenic centers of Daphlongeranine B, including one fully substituted carbon atom already in place.

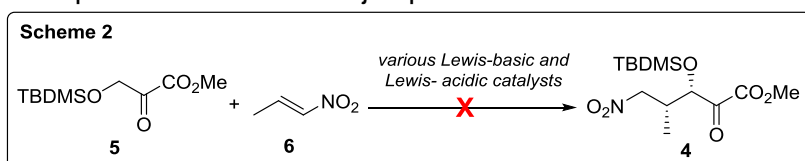


1.1 Attempts towards a Michael-addition reductive cyclisation strategy for the synthesis of XX

Initial studies as proposed in our fellowship application were centered on a catalytic Michael-addition – cyclisation strategy for elaborating nitrone **3**, an important intermediate en route to spirocycle **2** (Scheme 1). To this end, α -ketoester **5** and nitropropene **6** were prepared according to literature procedures⁴ and



subjected to a range of Lewis-acidic and Lewis-basic catalysts. Disappointingly, in my hands, none of the evaluated conditions proved successful, as formation of the desired γ -nitro ketone **4** could not be achieved under any of the applied conditions.⁵ Instead, the formation of oligomers, as well as 1,2-silyl migration in the ketoester coupling partner **5** proved to be the major problems associated with this strategy. It might be worth mentioning, that in existing related reports,⁶ a successful catalytic Michael-addition between a β -oxygenated α -ketoester and nitropropene (or similar aliphatic nitroolefins) has not yet been reported to the best of our knowledge.



¹ For a comprehensive review on *Daphniphyllum* alkaloids, see: Kobayashi, J. i.; Kubota, T., *Nat. Prod. Rep.* **2009**, *26*, 936.

² For some recently completed total syntheses of *Daphniphyllum* alkaloids, see: a) Weiss, M. E.; Carreira, E. M., *Angew. Chem. Int. Ed.* **2011**, *50*, 11501. b) Lu, Z.; Li, Y.; Deng, J.; Li, A., *Nat. Chem.* **2013**, *5*, 679. For some recent synthetic approaches to *Daphniphyllum* alkaloids, see: a) Darses, B.; Michaelides, I. N.; Sladojevich, F.; Ward, J. W.; Rzepa, P. R.; Dixon, D. J., *Org. Lett.* **2012**, *14*, 1684. b) Coldham, I.; Burrell, A. J. M.; Guerrand, H. D. S.; Oram, N., *Org. Lett.* **2011**, *13*, 1267. c) Xu, C.; Liu, Z.; Wang, H.; Zhang, B.; Xiang, Z.; Hao, X.; Wang, D. Z., *Org. Lett.* **2011**, *13*, 1812. d) Sladojevich, F.; Michaelides, I. N.; Benjamin, D.; Ward, J. W.; Dixon, D. J., *Org. Lett.* **2011**, *13*, 5132. e) Ikeda, S.; Shibuya, M.; Kanoh, N.; Iwabuchi, Y., *Org. Lett.* **2009**, *11*, 1833. f) Denmark, S. E.; Baiazitov, R. Y., *J. Org. Chem.* **2006**, *71*, 593. g) Sole, D.; Urbaneja, X.; Bonjoch, J., *Org. Lett.* **2005**, *7*, 5461.

³ Li, C.-S.; Di, Y.-T.; He, H.-P.; Gao, S.; Wang, Y.-H., et al., *Org. Lett.* **2007**, *9*, 2509.

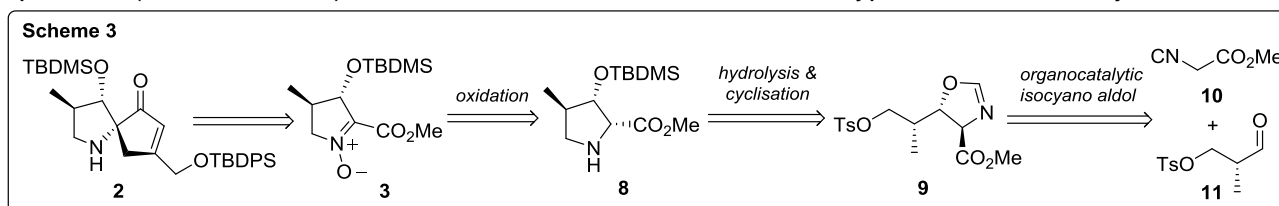
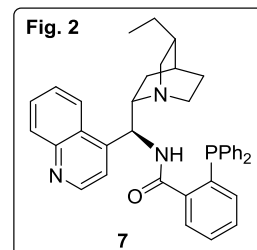
⁴ a) For preparation of ketoester, see: Peng, Z.-H.; Woerpel, K. A., *Org. Lett.* **2002**, *4*, 2945. b) For preparation of nitropropene, see: Peltier, H. M.; Ellman, J. A., *J. Org. Chem.* **2005**, *70*, 7342.

⁵ Catalysts evaluated involve: Ni(II), Urea (e.g. Takemoto-type), Thiourea (Schreiner-type), Proline, NEt₃

⁶ a) Xu, Y.; Matsunaga, S.; Shibasaki, M., *Org. Lett.* **2010**, *12*, 3246. b) Nakamura, A.; Lectard, S.; Hashizume, D.; Hamashima, Y.; Sodeoka, M., *J. Am. Chem. Soc.* **2010**, *132*, 4036. c) Baslé, O.; Raimondi, W.; Duque, M. d. M. S.; Bonne, D.; Constantieux, T.; Rodriguez, J., *Org. Lett.* **2010**, *12*, 5246.

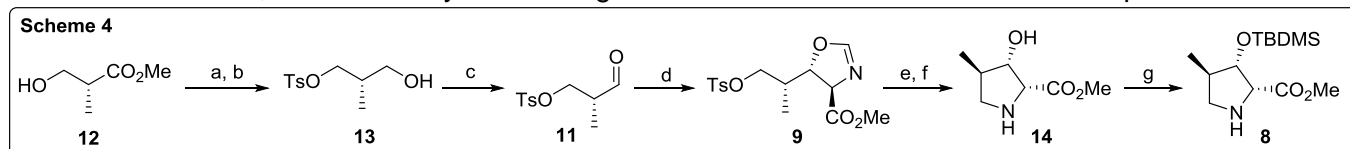
1.2 Development and optimization of a synthetic route towards highly functionalized pyrrolidine **8**

Given the failure of our originally proposed strategy, we quickly turned our attention to identifying an alternative route to key intermediate **2**. Our retrosynthetic analysis is shown in Scheme 3: the spirocyclic enone **2** should be accessible in four steps from **3**, including a [3+2] cycloaddition, ketone synthesis, N-O bond cleavage and an olefination reaction (see also Scheme 6). Synthesis of the nitron **3** by oxidation of the corresponding pyrrolidine is expected to be feasible, given the results on a very similar model system.⁷ For the key carbon-carbon bond forming event, we envisaged an catalytic isocyanoacetate aldol reaction between enantiomerically pure aldehyde **11** and methyl isocyanoacetate (**10**) using Ag₂O in combination with bifunctional organocatalyst **7**,⁸ which would be followed by hydrolysis of the resulting oxazoline and subsequent cyclisation. The starting materials for our planned approach are either commercially available (in case of **10**), or conveniently accessible in few steps from commercially available compounds (in case of **11**). Therefore, in the ideal case, we hypothesized densely functionalized



pyrrolidine **8** should be accessible in only four steps from simple and commercially available compounds as a single diastereomer in high chemical yield. Moreover, the implementation in a complex molecule synthesis would clearly highlight the synthetic potential of a highly stereoselective catalytic isocyanoacetate aldol reaction, which has been developed in the Dixon group (see ref. 7).

Our synthesis starts from commercially available (*R*)-(-)-Roche Ester (**12**, Scheme 4). Tosylation under standard conditions,^{9,10} followed by reduction gave rise to alcohol **13** in 89% over 2 steps.



a) TsCl (1.2 equiv), NEt₃ (1.3 equiv), DMAP (0.15 equiv), DCM, 0° C to r.t., 15h, 90%; b) DIBALH (2.5 equiv), THF, -78° C to r.t., 3h, 99%; c) SO₃-pyridine complex (2.9 equiv), DIPEA (2.9 equiv), DMSO, DCM, 0° C, 80min; d) methyl isocyanoacetate (**10**, 1.3 equiv), **7** (10 mol%), Ag₂O, EtOAc, 4A molecular sieves, -40° C to -20° C, 22h; e) HCl in MeOH (dry), 3h; f) sat. aq. K₂CO₃ solution, CHCl₃/iso-propanol (3:1), r.t., 15 min *then* extraction; g) TBSOTf (2.5 equiv), 2,6-lutidine (5 equiv), DCM, 0° C to r.t., 14h, 32% over 5 steps, *single diastereomer*.

For the synthesis of the aldehyde **11**, we were taking advantage of a Parikh-Doering oxidation using SO₃-pyridine complex.¹¹ This procedure was efficiently performed on up to 1 g scale. After performing an aqueous workup, crude **11** was sufficiently pure and because of its instability,¹² it was directly subjected to the subsequent isocyanoacetate aldol reaction. Other methods for the synthesis of aldehyde **11** involve direct reduction from the tosylated ester,¹³ IBX oxidation, as well as TEMPO¹⁴ oxidation of **13**. These methods have been evaluated and were found to be inferior in terms of reproducibility and scalability, as well as regarding the grade of purity of the crude aldehyde. Gratifyingly, reacting **11** with methyl isocyanoacetate (**10**) in ethyl acetate under silver/bifunctional organocatalysis¹⁵ resulted in a

⁷ These initial studies have been carried out by Eddy Kallstrom in the Dixon group.

⁸ Sladojevich, F.; Trabocchi, A.; Guarna, A.; Dixon, D. J., *J. Am. Chem. Soc.* **2011**, *133*, 1710.

⁹ Holmes, A. B.; Smith, A. L.; Williams, S. F.; Hughes, L. R.; Lidert, Z., et al., *J. Org. Chem.* **1991**, *56*, 1393.

¹⁰ The tosylate ester is known, see: Kummer, D. A.; Brenneman, J. B.; Martin, S. F., *Org. Lett.* **2005**, *7*, 4621.

¹¹ Evans, D. A.; Ripin, D. H. B.; Halstead, D. P.; Campos, K. R., *J. Am. Chem. Soc.* **1999**, *121*, 6816.

¹² The aldehyde **11** is very unstable, especially upon exposure to silica gel. At ambient temperature and open to the air, it decomposes within less than one hour. In EtOAc solution at -40° C, however, it is stable for at least three hours.

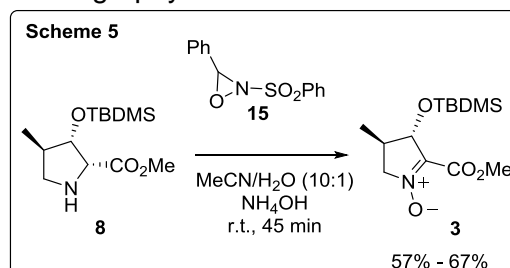
¹³ Aïssa, C.; Riveiros, R.; Ragot, J.; Fürstner, A., *J. Am. Chem. Soc.* **2003**, *125*, 15512.

¹⁴ Chany, A.-C.; Casarotto, V.; Schmitt, M.; Tarnus, C.; Guenin-Macé, L., et al., *Chem. Eur. J.* **2011**, *17*, 14413.

¹⁵ Sladojevich, F.; Trabocchi, A.; Guarna, A.; Dixon, D. J., *J. Am. Chem. Soc.* **2011**, *133*, 1710.

clean formation of oxazoline **9**.¹⁶ Cinchonidine-derived catalyst **7** in combination with catalytic amounts of Ag₂O provided the desired product in good diastereomeric ratio (10:1 by ¹H-NMR).¹⁷ Remarkably, there is no need for further purification as the crude oxazoline **9** is obtained sufficiently pure after filtration over a short pad of silica gel.¹⁸ Treatment of this compound with a freshly prepared solution of HCl in dry MeOH¹⁹ led to the expected opening of the heterocycle, which upon subjection to mildly basic conditions (i.e. a saturated aqueous K₂CO₃ solution) afforded the desired pyrrolidine **14**.^{20, 21} Exposing crude **14** to an excess of TBSOTf and 2,6-lutidine in dry DCM ultimately led to the desired protected pyrrolidine **8** as a single diastereomer²² in a good chemical yield of 32% over five steps.²³ Pleasingly, the whole sequence only requires one final purification by column chromatography.

The oxidation of the pyrrolidine **8** to the corresponding nitron proved non-trivial. Our current best conditions take advantage of Davis oxaziridine **15**²⁴ as oxidant and give rise to the nitron **3** in acceptable chemical yields of 57% - 67% (Scheme 5). Attempts to use other oxidants have so far proven to be less efficient and/or providing mixtures of regioisomeric nitrones.²⁵ Nevertheless, endeavours to further optimize this transformation are currently ongoing in the Dixon group.



In conclusion, an efficient synthetic procedure for the synthesis of densely functionalized pyrrolidine **8** could be established during this fellowship. Our approach gives rise to the desired product as a single diastereomer in good chemical yield. Notably, the five-step sequence from alcohol **13** to the final product **8** only requires one single purification by column chromatography. Since the developed sequence is scalable,²⁶ it will provide the required quantities of material for consecutive studies. These are currently ongoing in the Dixon group and are expected to culminate in the first total synthesis of our target molecule, Daphlongeranine B in due course.

¹⁶ Most conveniently, the reaction was performed on 500 mg scale (referring to the alcohol **13**). Batches of less than 100mg alcohol were found to give non-reproducible diastereomeric ratios, most probably due to the limited weighing accuracy with respect to Ag₂O (increasingly unselective background reaction).

¹⁷ Using tbutyl isocynoacetate, an improved d.r. of 30:1 in favour of the desired isomer could be obtained. Despite this result, we chose to use its methyl counterpart, as we expect it to be beneficial with respect to the ketone formation later on in the synthesis.

¹⁸ The crude oxazoline is unstable, especially upon prolonged exposure to silica gel. After performing a quick filtration followed by concentration under reduced pressure, crude **9** is immediately redissolved in dry MeOH, cooled to 0° C and subjected to methanolysis.

¹⁹ Prepared by mixing SOCl₂ with dry MeOH under N₂.

²⁰ After methanolysis of the oxazoline followed by evaporation, MS indicates almost exclusively the open-chain, d-tosyloxy amine. After treatment with saturated aqueous K₂CO₃ solution, MS indicates exclusively pyrrolidine **14**.

²¹ Since the unprotected pyrrolidine is very polar, extraction with CHCl₃/PrOH (3:1) needs to be carried out very thoroughly and using only saturated aqueous solutions, in order not to lose material at this stage.

²² The assignment of the relative stereochemistry was performed based on an NOE analysis of the major diastereomer. The minor diastereomer could not be isolated.

²³ That corresponds to an average yield of 80% per step.

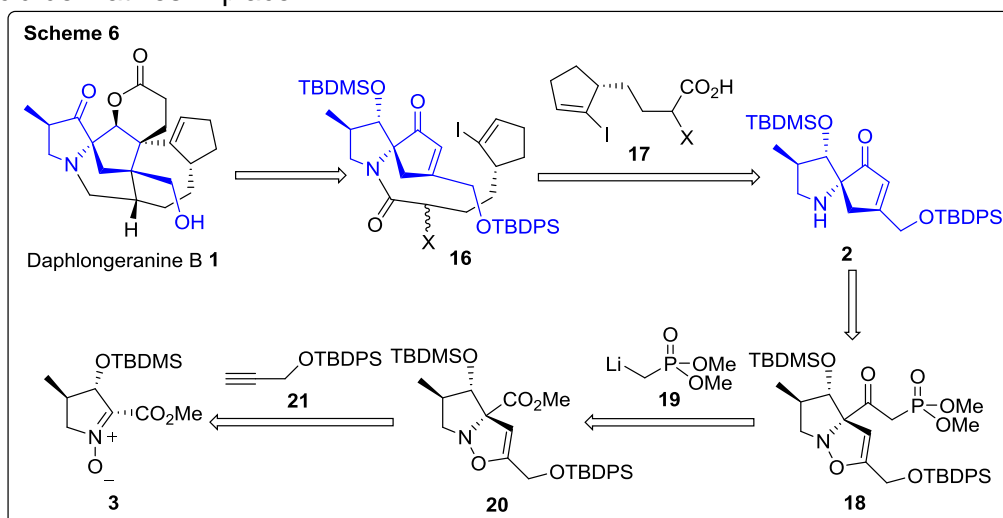
²⁴ Davis, F. A.; Chen, B. C., *Chem. Rev.* **1992**, *92*, 919.

²⁵ Oxidants examined include: Oxone, MeReO₃/UHP, MeReO₃/H₂O₂, Na₂WO₄/H₂O₂.

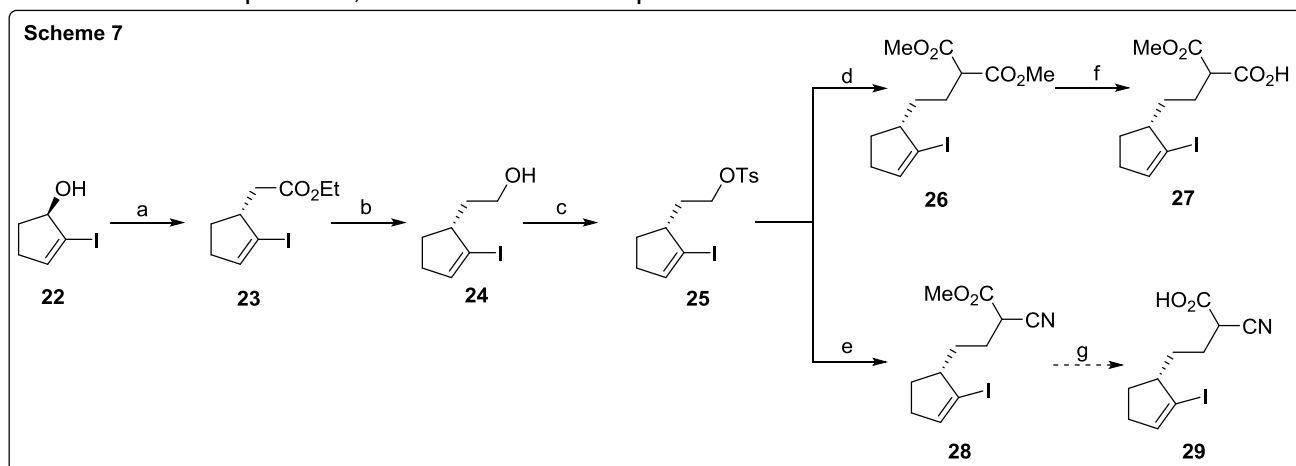
²⁶ Most conveniently, the reaction was performed on 500 mg scale (referring to the alcohol **13**). The sequence was performed twice on 1g scale, however obtaining inferior chemical yields (22% overall). I found that exceeding the 500 mg scale, the handling of the unstable compounds (evaporation, extraction) becomes more and more inconvenient.

1.2 Synthesis of chiral side chains bearing different electron withdrawing groups

As depicted in Scheme 6, our retrosynthetic analysis of our target Daphlongeranine B (**1**) involves a chiral side chain of general structure **17** being coupled to spirocyclic enone **2**. Also, our synthetic plan involves a Michael addition of an enolate (generated from the amide in **16**) onto the spirocyclic Michael acceptor. Taking this into account, we considered it as beneficial to have a set of different acceptor substituted acid derivatives in place.



In the course of this fellowship, syntheses of derivatives **26** and **28**, bearing two different electron-withdrawing groups alpha to the carboxylic acid function, were developed. Both syntheses start from enantiomerically pure alcohol **22**.²⁷ A Johnson-Claisen rearrangement using triethyl orthoacetate gives rise to ester **23** in 60% yield, which is then reduced to alcohol **24**. Transformation into a good leaving group using TsCl affords tosylate **25** in high yield, which can then be transformed into malonic acid derivatives **26** and **28**, respectively. Under S_N2 -conditions, dimethyl malonate derivative **26** is accessible in 87% chemical yield, whereas the corresponding methyl malonomononitrile **28** is obtained in 39%. A selective monohydrolysis of diester **26**²⁸ gives rise to β -keto acid **27** in 66%. Hydrolysis of the mononitrile **28** was shown to be possible, however it was not optimized.



a) $\text{MeC}(\text{OEt})_3$, 2-nitrophenol (5 mol %), 120°C , 14h, 60%; b) LiAlH_4 (2.5 equiv), Et_2O , 0°C to r.t., 2h, 89%; c) TsCl (1.4 equiv), NEt_3 (9.4 equiv), DMAP (0.1 equiv), DCM, r.t., 16h, 88%; d) Dimethyl malonate (3.0 equiv), KO^tBu (3.0 equiv), KI (1.0 equiv), DMF/THF (1:1), r.t., 4d, 87%; e) Methyl 2-cyanoacetate (3.0 equiv), KO^tBu (3.0 equiv), KI (1.0 equiv), DMF/THF (1:1), r.t., 4d, 39%; f) Aq. KOH (1M) (0.9 equiv), MeOH, r.t., 3h, 39%. g) LiOH (5.0 equiv), MeOH/ H_2O (1:1), r.t., 6h, 44%, unoptimised

²⁷ Kabat, M.; Kiegiel, J.; Cohen, N.; Toth, K.; Wovkulich, P. M., et al., *Tetrahedron Lett.* **1991**, 32, 2343.

²⁸ Hong, S.; Lee, J.; Kim, M.; Park, Y.; Park, C., et al., *J. Am. Chem. Soc.* **2011**, 133, 4924.

2. SUMMARY OF THE PROGRESS OF THE RESEARCH TRAINING

2.1 Attendance of seminars and lectures

During my fellowship, I have had the opportunity to attend the first "Chemistry Careers Conference", held by the department of Organic Chemistry. In this one day seminar, I could learn about the different career pathways for researchers, both in academia and industry. Also, I had the opportunity to participate in a workshop entitled "managing your mind". Furthermore, I attended numerous lectures held by leading academic researchers from all over the world, providing me with the opportunity to broaden my chemistry-related knowledge significantly. One highlight of the academic year was the Newton Abraham Symposium, including a lecture by Nobel Prize awardee Prof. Richard Schrock entitled "Olefin Metathesis: A Long-Lived Reaction".

2.2 Research skills and techniques

This Marie Curie fellowship provided me with the opportunity to develop new skill and abilities, which complement those, I already possessed. In particular, I developed new skills related to the field of total synthesis, analytical methodology (NMR, HPLC, MS, IR), organic synthesis methods and techniques (especially reaction scale-up, asymmetric synthesis). The fellowship project in combination with the outstanding research infrastructure at Oxford University was an ideal training environment for me to develop as a research scientist.

2.3 Communication and professional skills

Apart from the scientific part of the project, I had the opportunity to develop my abilities as a communicator and professional research scientist. Research results, progress and the work plan for the near future was discussed on a weekly basis. The presentation of research updates in front of the whole group including my supervisor Prof. Dixon was done on a weekly basis. These group meetings provided the opportunity for in-depth discussions of my research involving all post-doctoral and post-graduate co-workers of the Dixon group. In addition, I had the opportunity to discuss strategy and direction of my project in one-to-one meetings with Prof. Dixon on a weekly basis, ensuring progress and smooth-running of the fellowship.