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Towards the total synthesis of the potent fungicide amphidinol 3

Pol Karier a obtenu son doctorat en sciences le 14 décembre 2016.
En hommage au promoteur de sa thèse, le Professeur I. Markó, inopinément décédé le 31 juillet 2017, il lui dédie ce texte, résumé de sa thèse de doctorat.

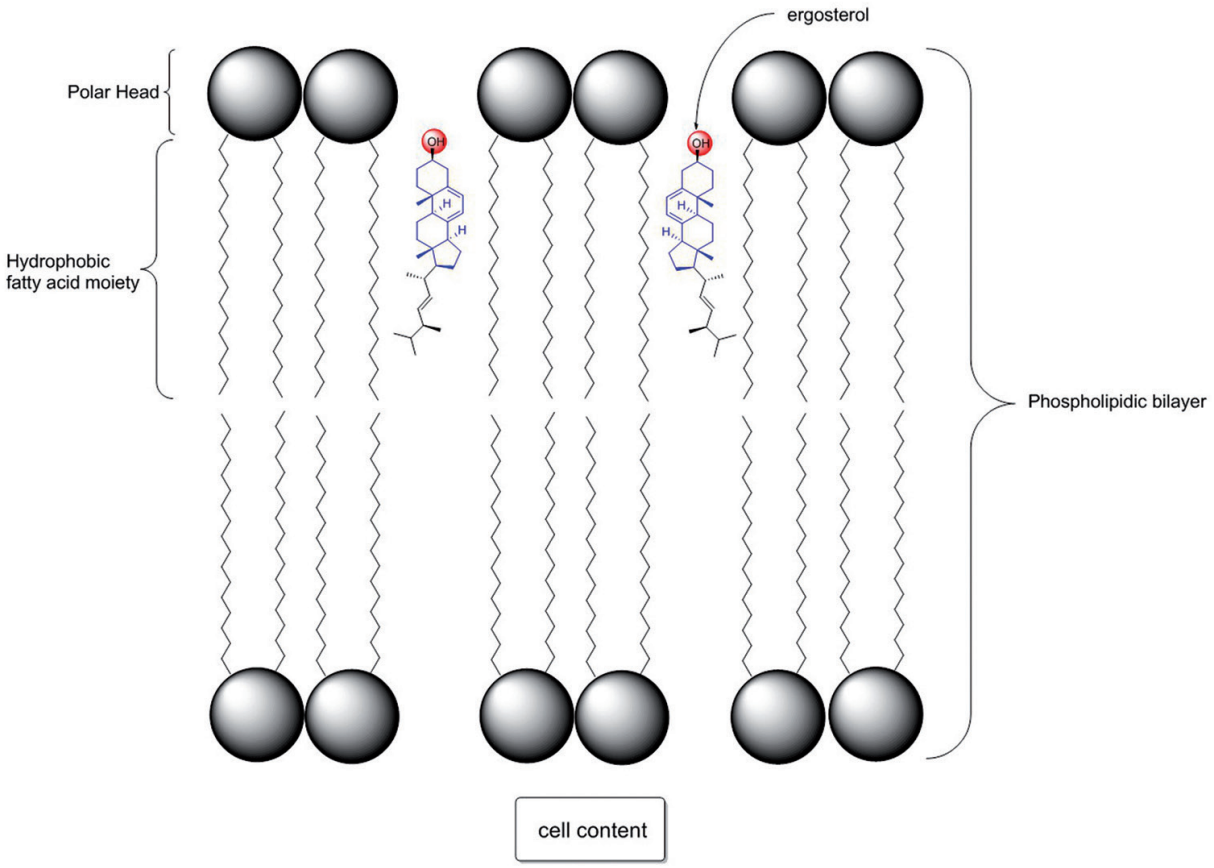
Abstract
Amphidinols are members of a family of marine toxins that are produced by the microplanktonic unicellular organisms Amphidinium klebsii and Amphidinium carterae. It is suggested that these molecules serve as chemical defence against other bottom-dwelling organisms, with which the algae competes for living territory and nutrition. Amphidinols exhibit a variety of biological properties, including antifungal, hemolytic, cytotoxic and ichthyotoxic actions. The third member of the family, amphidinol 3 ***1*** *was discovered in 1996 and is, to date, the only amphidinol whose three dimensional structure is fully established (Figure 1). The synthesis of the C15-C30 domain, as well as the preparation of the C31-C40 trans-configurated tetraydropyran will be discussed throughout this work.*

Key words
Fungicide, Amphidinol, Sterol, Dinoflagellate, Marine Toxins.

1. Introduction

The fluidity of cell membranes is strongly modulated by the presence of sterols in the phospholipid bilayer [1]. The ***alcohol residue*** of the sterol interacts with the polar head of the phospholipids, whereas the ***bulky sterane*** unit prefers to lay embedded in the hydrophobic fatty-acid moiety (Scheme 1). Besides influencing the physicochemical properties of biological membranes, sterols can be used by the cell as precursors for secondary messengers or as building blocks for fat-soluble vitamins. A plethora of antifungal agents that are in current medical use, including amphotericin B and filipin interact in some way or another with sterols or with sterol metabolism [2]. Toxicity problems arise when these drugs exhibit poor selectivity towards ergosterol, the main sterol component of fungi cell membranes, compared to cholesterol, the major zoosterol. Poor selectivity i.e. high cytotoxicity is still one of the major constraints regarding the clinical use of compounds exhibiting sterol-dependant antifungal mechanisms.

In the quest to conquer new antifungal compounds, the curiosity of the scientific community was attracted by the microalgal dinoflagellates *Amphidinium klebsii* and *Amphidinium carterae* in 1991 [3]. These marine protists produce around 20 known amphiphilic architectures exhibiting a variety of biological properties, including antifungal, antidiatom, hemolytic,



Scheme 1. Representation of a phospholipidic bilayer containing ergosterol. In red: the alcohol residue; in blue: the bulky sterane

cytotoxic and ichthyotoxic actions. These fearsome natural products were named amphidinols and amongst them, amphidinol 3 exhibits the strongest antifungal activities (Scheme 2) [4]. Amphidinol 3 is also the only member whose three dimensional structure is fully established.

In natural environment, it is suggested that amphidinols serve as chemical defence against other bottom-dwelling organisms with which the producing dinoflagellates compete for living territory and nutrition. The bioactivities derive from the disturbance of the arrangement of the lipid bilayer of the targeted cells [5]. The membrane disrupting properties are dependant and potentiated by the presence of membrane sterols [6]. There is reason to assume that amphidinols bind to the sterol present in the biological membranes. These amphidinol·sterol complexes then further associate, forming transmembrane pores of 2-3 nm that lead to leakage of cell content and eventually to cell death (Scheme 2) [7].

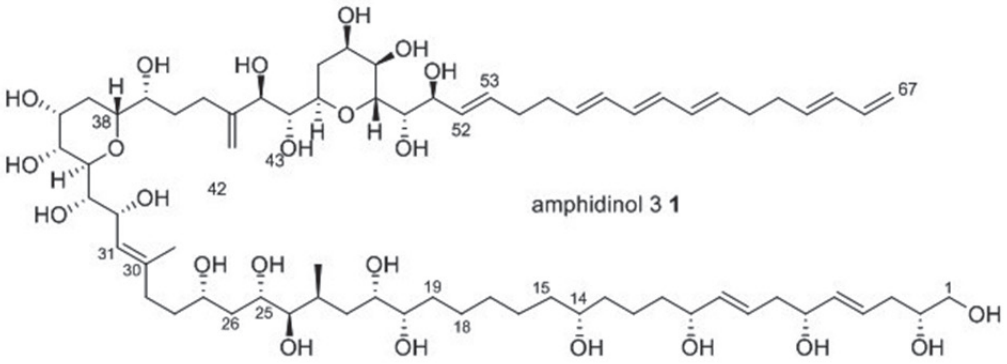
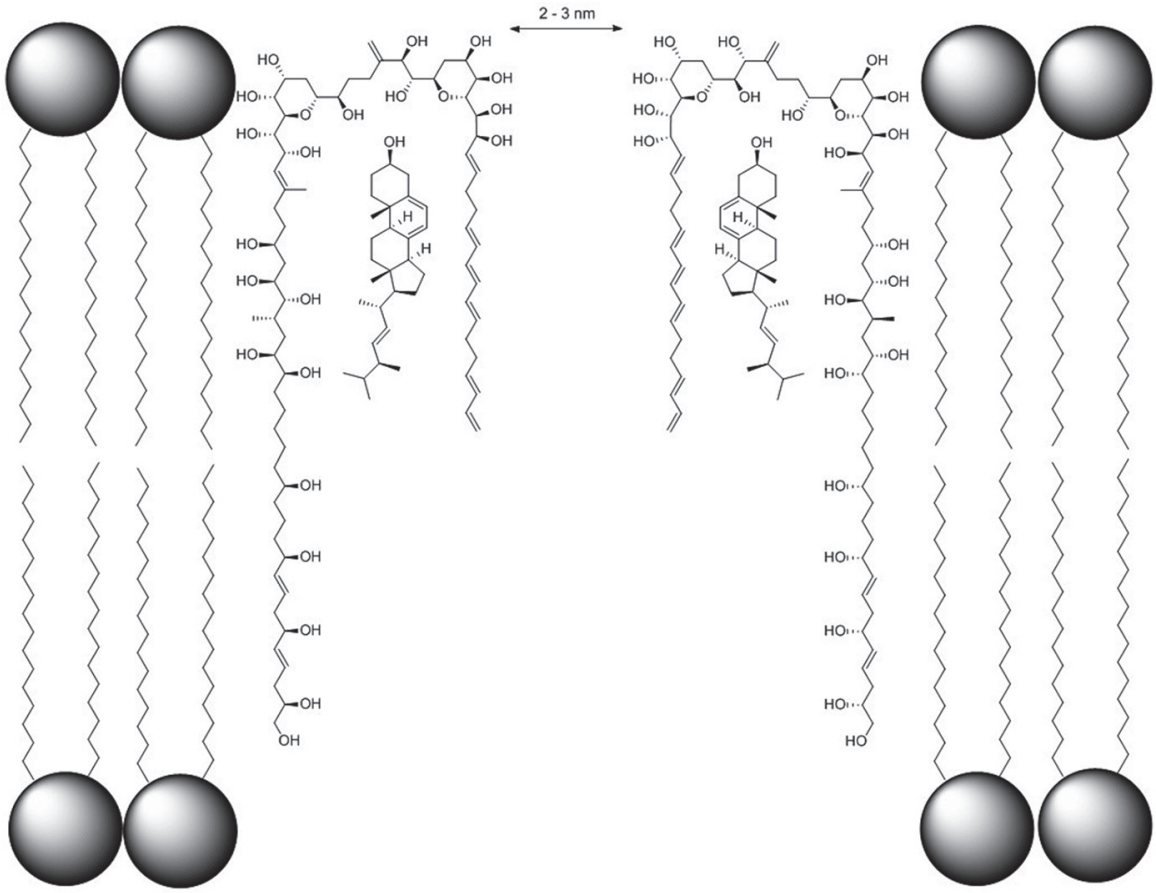


Figure 1. Three dimensional structure of amphidinol 3



Scheme 2. Sterol dependant pore formation mechanism of amphidinol 3

Due to the intriguing biological properties of amphidinol 3, combined to the challenging structure encompassing 25 chiral centres and 10 unsaturations, our group became interested in the chemical synthesis of this natural product [8]. In this communication we want to describe chiefly our efforts towards the total synthesis of amphidinol 3.

2. Retrosynthesis

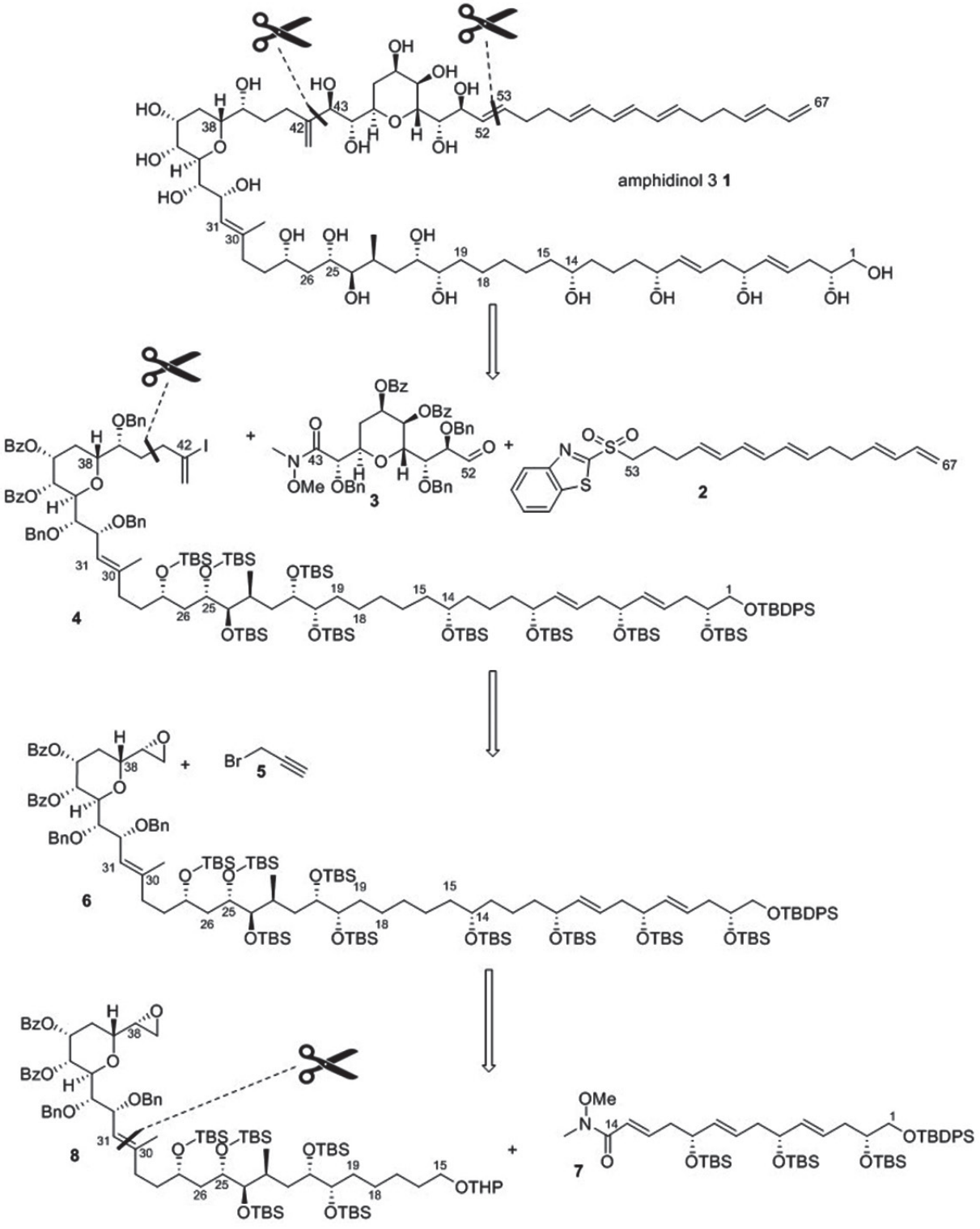
To ease the synthesis of this complex molecule, the natural product is dissected into smaller subunits. Scheme **3** highlights our initial disconnection strategy of amphidinol 3 **1**. Our retrosynthetic analysis leads to the polyene **2**, the tetrahydropyran **3** and the C1-C42 fragment **4**. The synthesis of **2** was already described in our laboratory previously [9]. The synthesis of tetrahydropyran **3** lies beyond the scope of this thesis; however, its preparation can rely on methodologies developed in our laboratory. Upon a few basic steps, epoxide **6** is a logical precursor of intermediate **4**.

We envisioned that subsector **6** could be derived from triene **7** whose synthesis was described previously [10], and from the complex fragment **8**. The strategy to establish the C14-C15 junction would be entirely based upon an approach known in literature [11], enhancing the chances for its success. The main goal of this thesis is to assemble the central fragment **8** of amphidinol 3.

3. Results

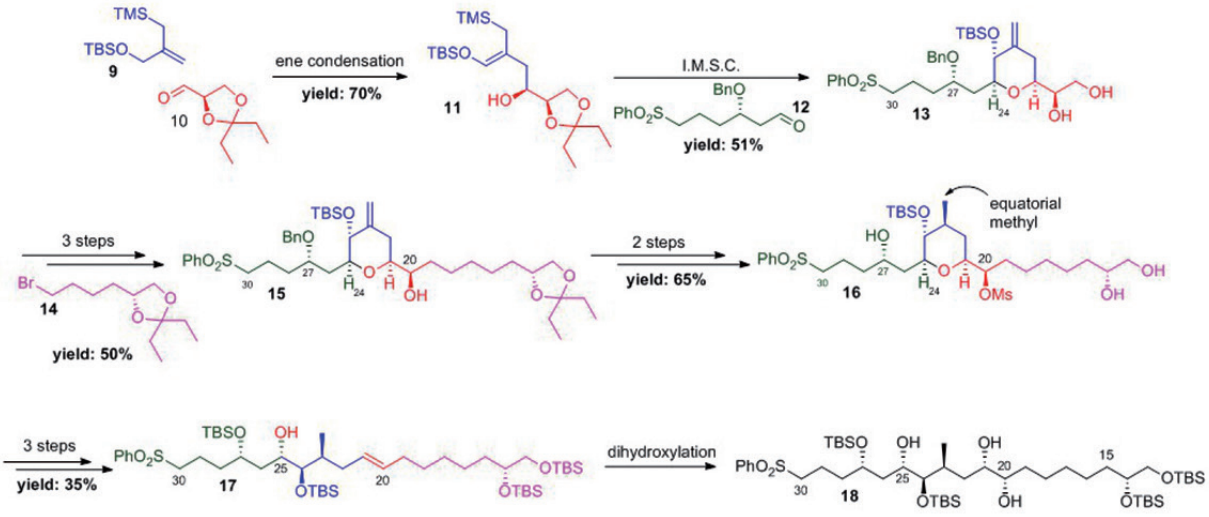
3.1. Synthesis of the C15-C30 substructure

The synthesis of fragment **8** starts from the building blocks **9** and **10** that are submitted to a condensation developed previously in our laboratory (Scheme 4) [12]. The following intramolecular Sakurai condensation (I.M.S.C.) allows to unify fragment **11** with aldehyde **12** [13]. The eastern lateral chain **14** is then introduced. After 11 linear operations, the unsaturated compound **15** is isolated in 15% overall yield. Two



Scheme 3. Retrosynthetic analysis

further operations, including a heterogeneous hydrogenation deliver the saturated product **16**. To complete the sequence, the oxygenated centre in the position C20 is first transformed into a halogenated equivalent, setting thereby the stage for the original reductive fragmentation that furnishes eventually the entire C15-C30 backbone of the natural product (compound **17**). A final dihydroxylation installs the remaining chiral centres in fragment **18**.



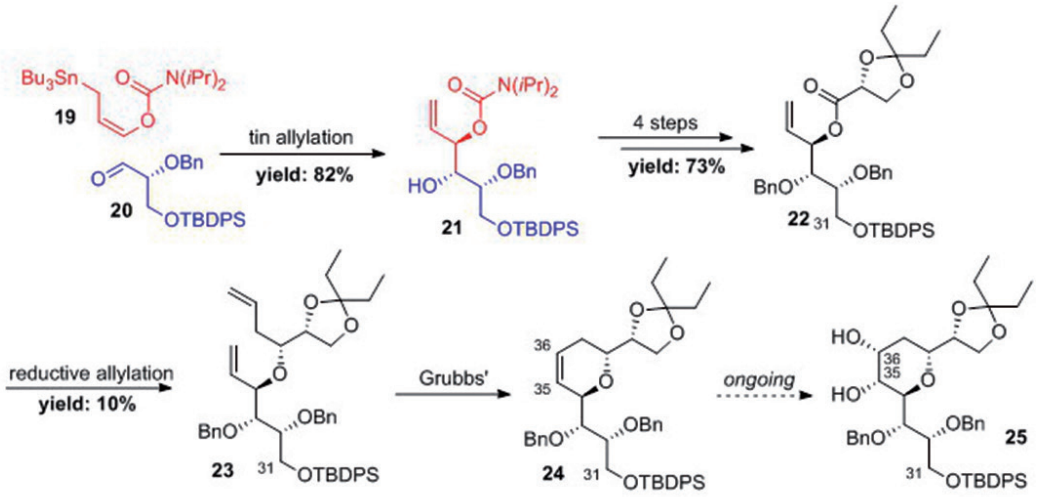
Scheme 4. Synthesis of the C15-C30 subunit

3.2. Synthesis of the C31-C40 substructure

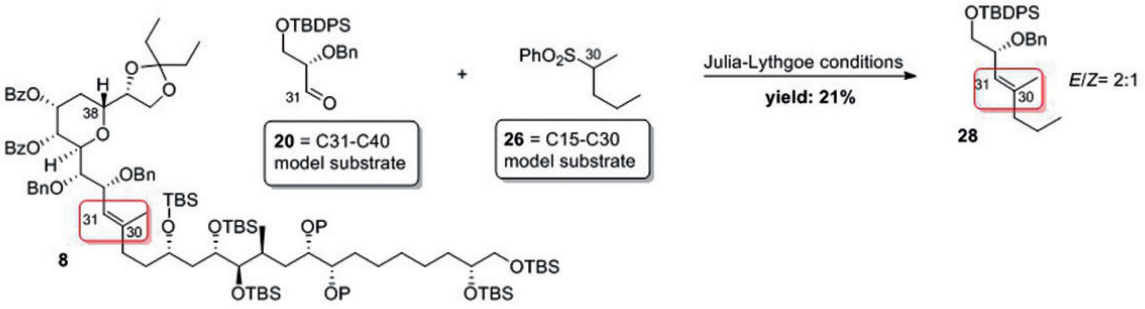
With the C15-C30 fragment in hand, we investigated the synthesis of the C31-C40 tetrahydropyran **25** (Scheme 5) [14]. The synthesis of this subunit relies upon an in-house developed allylation implying allylstananne **19** and aldehyde **20**. A few decoration steps transform the intermediate **21** into the ester **22** that is further submitted to a reductive allylation protocol [15]. Diene **23** is further elaborated into dihydropyran **24**, lacking solely the diol functionality in the C35-C36 position. With the two fragments **18** and **24** in hand, we became interested in the generation of the C30-C31 double bond.

3.3. Connection of the C15-C30 and the C31-C40 substructures

The formation of the C30-C31 double bond with concomitant union of the subunits of type **18** and **24** was studied on the model substrates **20** and **26** (Scheme 6). The results show that this transformation is possible, but that further optimization is required.



Scheme 5. Synthesis of the C31-C40 dihydropyran



Scheme 6. C30-C31 double bond generation as planned on substrates 18 and 24 and on model substrates 20 and 26.

4. Conclusions

There is no doubt that nature is the most skillful and imaginative chemist in natural product synthesis, stitching together extremely beautiful molecular architectures. The scientific community has recognized the nature as a rich source of natural products that exhibit extremely powerful medicinal behaviors. In this work, marine dinoflagellates are used as role models for the development of new antifungal compounds. During this thesis, we showed that our approach is capable to deliver advanced and complex intermediates of the natural product. Most of the fragments of amphidinol 3 have been prepared by our group. However, no efforts could be centralized around the connection of these subunits to sew together the final natural product.

Références

[1] E. J. Dufourc, *J. Chem. Biol.* **2008**, *1*, 63.
[2] a) B. C. Wilcock, M. M. Endo, B. E. Uno, M. D. Burke, *J. Am. Chem. Soc.* **2013**, *135*, 8488. b) P. A. Orlandi, P. H. Fishman, *J. Cell. Biol.* **1998**, *141*, 905.
[3] G. K. Paul, N. Matsumori, K. Konoki, M. Sasaki, M. Murata, K. Tachibana, in 'Harmful and Toxic Algal Blooms' **1996** Structure of amphidinol 3 and its cholesterol-dependent membrane perturbation: a strong antifungal metabolites produced by dinoflagellate, Amphidinium *klebsii*. In: T. Yasumoto, Y. Oshima, Y. Fukuyo, [eds.], 503. Intergovernmental Oceanographic Commission of UNESCO, Sendai.
[4] M. Murata, S. Matsuoka, N. Matsumori, G. K. Paul, K. Tachibana, *J. Am. Chem. Soc.* **1999**, *121*, 870.
[5] G. K. Paul, N. Matsumori, K. Konoki, M. Murata, K. Tachibana, *J. Mar. Biotechnol.* **1997**, *5*, 124.
[6] R. T. Swasono, R. Mouri, N. Morsy, N. Matsumori, T. Oishi, M. Murata, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2215.
[7] M. Murata, N. Matsumori, S. Matsuoka, T. Houdai, *Biochim. Biophys. Acta* **2004**, *1667*, 91.
[8] Review covering the work until the end of 2013: C. Bensoussan, N. Rival, G. Hanquet, F. Colobert, S. Reymond, J. Cossy, *J. Nat. Prod. Rep.* **2014**, *31*, 468.
[9] a) J. Cossy, T. Tsuchiya, L. Ferrié, S. Reymond, T. Kreuzer, F. Colobert, P. Jourdain, I. E. Markó, Synlett 2007, 14, 2286. b) J. Cossy, T. Tsuchiya, L. Ferrié, S. Reymond, T. Kreuzer, F. Colobert, P. Jourdain, I. E. Markó, Synlett 2007, 15, 2351.
[10] S. BouzBouz, J. Cossy, *Org. Lett.* **2001**, *3*, 1451.
[11] J. Vicente, J. R. Huckins, S. D. Rychnovsky, *Org. Lett.* **2007**, *9*, 4757.
[12] I. E. Markó, D. J. Bayston, *Tetrahedron Lett.* **1993**, *34*, 6595.
[13] a) A. Mekhalfia, I. E. Markó, *Tetrahedron Lett.* **1991**, *32*, 4779. b) A. Mekhalfia, I. E. Markó, H. Adams, *Tetrahedron Lett.* **1991**, *32*, 4783.
[14] C. G. Catrinescu, UCL PhD Thesis, **2014**.
[15] D. J. Kopecky, S. D. Rychnovsky, *J. Org. Chem.* **2006**, *65*, 191.