CITRUS NURSERY RESEARCH BOARD

Final Report, September 21, 2008

Project Title: Development of a short and efficient synthesis of the citrus leafminer pheromone blend

Project Leader: Jocelyn G. Millar

Background: We recently identified the two-component pheromone blend of citrus leafminer (Moreira et al., 2006), and in field trials in California, Florida, the Mediterranean basin, and Brazil, the pheromone has proven to be extremely attractive to male leafminers, with hundreds of leafminers caught per trap per night. Recent work in Florida has also shown that the pheromone has major potential for control of citrus leafminer by mating disruption; pheromone deployed at 1.5 grams/hectare reduced male moth orientation to pheromone by 98% and reduced flush infestation by larvae for >200 days (Stelinski et al. 2008).

However, manufacturing enough pheromone for large-scale deployment remains a major bottleneck in the continued development of pheromone-based control of this insect. The syntheses of the pheromone components that were developed during the identification of the pheromone are too long and too expensive to provide enough pheromone on a large scale. The object of this project was to devise and test shorter and more efficient syntheses of the leafminer pheromone components, so that it could be produced more economically.

Rationale:

We reasoned that it might be possible to both cut the costs and shorten the syntheses of the two pheromone components by using two complementary approaches. First, the citrus leafminer pheromone consists of two chemicals that differ only by one double bond (the major component has three double bonds, whereas the minor component has two). Thus, we reasoned that we might be able to shorten the overall synthetic steps required to make these compounds if we could develop a convergent synthetic route, whereby we could make both compounds simultaneously and in the same flask. Thus, we thought that we might be able to attach a building block A (which was part of the structure of both compounds), to two different building blocks B and C in one step in the same pot, with the ratio of B and C adjusted so that the blend of products AB and AC in the product mixture was equivalent to the blend in the insect's pheromone. If this strategy worked, we could cut the overall number of steps required to synthesize the two pheromone chemicals almost in half.

The second part of our strategy for shortening the synthesis of the pheromone components involved the use of a double-ended intermediate, with each end reacting sequentially in one pot, so that two sequential steps could be carried out in the same pot without having to purify the intermediate.

Over the past two years, we have made several concentrated attempts to bring these strategies to fruition, as follows. In the first attempt (Fig. 1), combining both strategies of using a double-ended reagent and simultaneously coupling the resulting shared fragment to the correct mixture of two different fragments required to complete the carbon skeletons of the two pheromone components in one pot, the chemistry did indeed work, but not even close to the selectivity or yields reported in the literature (Pohnert and Boland, 2000). The problem appears to lie with the double-ended reagent: all attempts at optimizing the two sequential couplings to provide reasonable yields of the desired products have failed, despite numerous variations of solvent, base, and temperature. In particular, the first coupling is nowhere near as selective or complete as claimed in previous reports, so that when the second two aldehyde fragments are added to the reaction mixture simultaneously, a mixture of all possible combinations of additions of the three aldehydes results. The two desired products are indeed there, but in relatively poor yield and mixed up with other coupling products (plus the triphenylphosphine oxide residue), making it virtually impossible to cleanly pull out the desired products on a preparative scale. It must also be emphasized that these attempts were carried out independently by two highly skilled postdoctoral chemists, both of whom have successfully synthesized a number of molecules with more difficult structures than those of the citrus leafminer. Rather, the problem appears to be intrinsic to the chemistry of the reagents and intermediates.

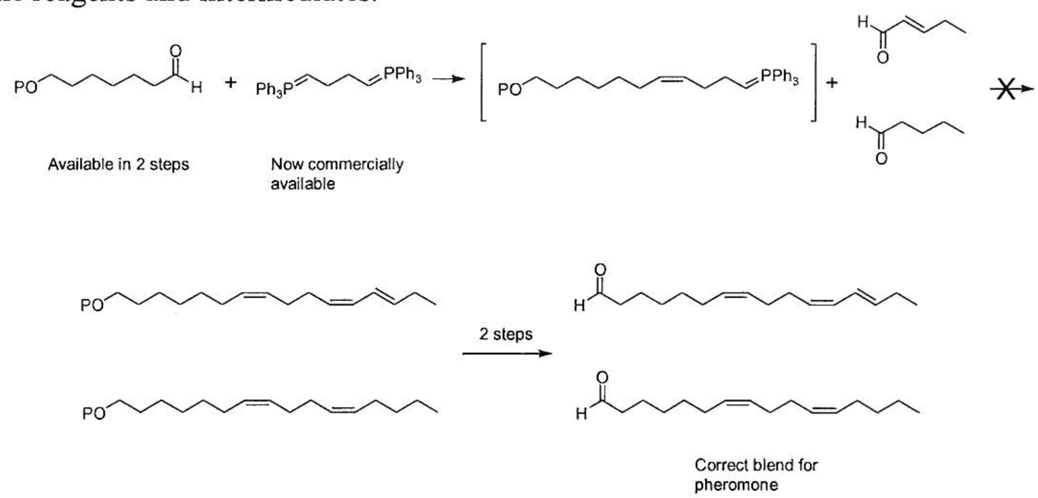

Figure 1. Attempted simultaneous synthesis of the two components of the citrus leafminer pheromone blend using a double-ended reagent, and attaching a shared fragment to two different fragments simultaneously

---

The second route that was attempted is not as convergent, but would still shave a number of steps off the published syntheses. Again, a key strategy was to use a double-ended reagent in a first coupling to generate an intermediate that is common to both the pheromone components, and then in the same pot, to couple this intermediate with the two different pieces which will complete the carbon skeletons of the two components of the pheromone, which can then be carried forward in the rest of the steps in tandem. In this case, the double-ended reagent is the dianion of propyne. The general strategy is shown in Fig. 2.

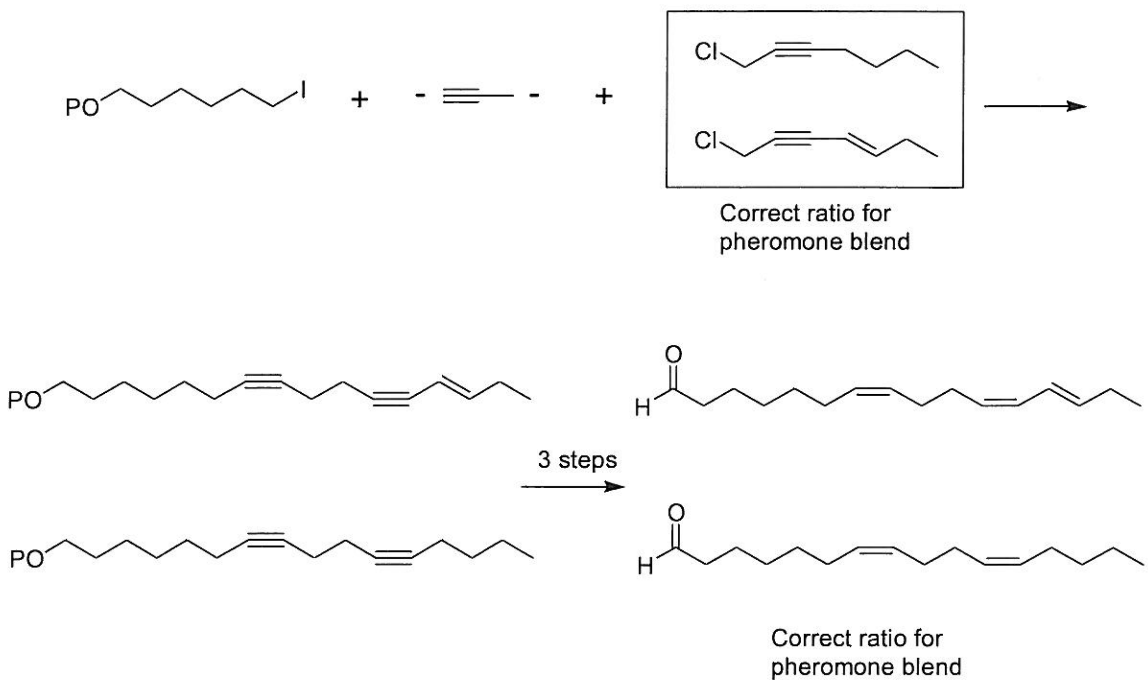

Fig. 2. Second possible route to produce the pheromone blend of citrus leafminer, using a double-ended reagent prepared from allene.

However, in practice, this synthetic route has also proven to be problematic, and consequently, we have backed off a step, and are working through this synthetic route with each of the two pheromone compounds independently, to make sure that the route will indeed work for each compound. Once we have verified that it is indeed possible to produce both compounds by this route, we will then try converging the two routes to cut the overall number of steps.

Thus, the synthesis of one of the pheromone components has been successfully carried through this reaction sequence, producing the penultimate intermediate for the desired pheromone compound in five steps overall from commercially available starting materials (Fig. 3).

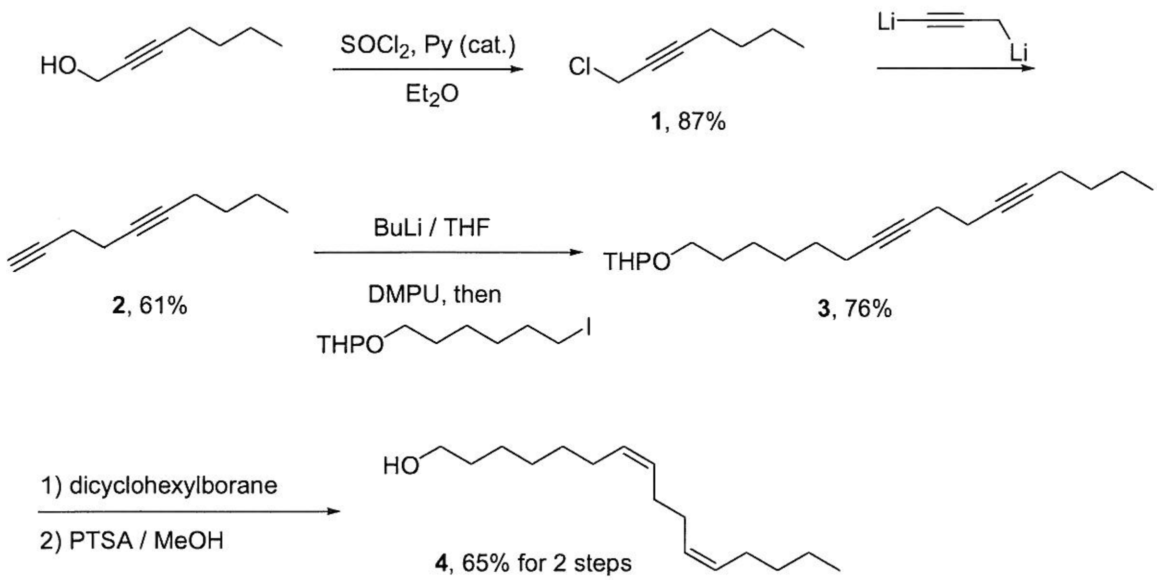

Fig. 3. New, shorter synthesis of (7*Z*,11*Z*)-hexadecadienol, the penultimate intermediate to (7*Z*,11*Z*)-hexadecadienal, one of the two components of the citrus leafminer pheromone.

The first steps of the same general series of reactions worked satisfactorily in the synthesis of the other pheromone component with three double bonds (Fig. 4), up to enediyne intermediate 8. Unexpectedly, coupling of this intermediate with a six-carbon segment to complete the chain has proven problematic, with the reaction mixture going to black tarry material from which little of the desired product can be recovered. We are currently experimenting with lower temperatures and different solvents to determine whether we can find a set of conditions that will favor the desired coupling reaction over the decomposition/polymerization that is producing the tarry material. Assuming that this troublesome step can be worked out, we do not anticipate any problems with the remaining two steps.

From there, the stage will then be set for careful merging of the two syntheses, with compounds 1 and 7 being carried forward in tandem, and subjected to the same sequence of reactions to finish the syntheses of both compounds in a ratio approximating the optimum ratio produced by the insects.

Overall, these synthetic strategies and syntheses have proven to be far more difficult to execute than is suggested by the literature, for both strategies described above. To date, we have expended time, effort, and monies far in excess of the original grant request, but we are determined to see this through to completion, with a shorter and more efficient synthesis of the pheromone blend than those currently available in the literature. Furthermore, the general concept of synthesizing two or more closely related pheromone components simultaneously rather than sequentially should be applicable to a variety of other insect pheromone blends.

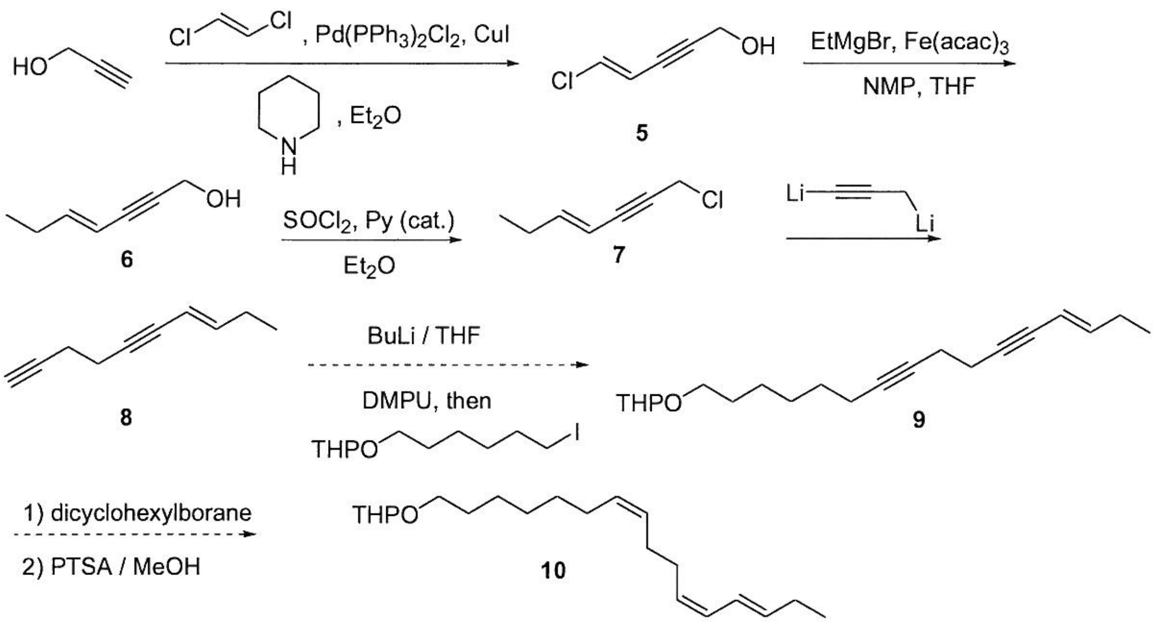

Fig. 4. Synthesis in progress for the second component of the citrus leafminer pheromone. The conversion of 8 to 9 is currently being optimized.

References cited:

Moreira, Jardel A.; McElfresh, J. Steven; Millar, Jocelyn G. 2006. **Identification, synthesis, and field testing of the sex pheromone of the citrus leafminer, *Phyllocnistis citrella.*** Journal of Chemical Ecology 32, 169-194.

Pohnert, Georg; Boland, Wilhelm. 2000. **Highly efficient one-pot double- Wittig approach to unsymmetrical (1Z,4Z,7Z)-homoconjugated trienes.** European Journal of Organic Chemistry 9, 1821-1826.

Stelinski, L. L.; Miller, J. R.; Rogers, M. E. 2008. **Mating Disruption of Citrus Leafminer Mediated by a Noncompetitive Mechanism at a Remarkably Low Pheromone Release Rate.** Journal of Chemical Ecology 34, 1107-1113.