Ph.D. Thesis Review

Ph.D. Thesis title: Synthesis of neuraminidase binders suitable for theranostics

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Carlos Berenguer has worked under my supervision for the past five years. His first and actually major project dealt with the preparation of sialomimetics that interact with the neuraminidase enzyme of the influenza virus. The beginning of this project was not easy and Mr. Berenguer struggled with the last two key steps of the synthetic route. He eventually attempted a few different approaches towards oseltamivir derivatives equipped with polar side-chains and, as is well documented by a short series of products resulting from this effort, he was successful. Unfortunately, pursuit of the first goal of the Thesis took two years and I decided to move forward regardless the fact that the first SAR cycle was barely accomplished. Here I have to admit that these two tough years, together with the eager supervisor's constant attention, turned this young colleague into an excellent chemist. I have come to realize it later, during the past eighteen months, when I was witness to two other projects conducted by Mr. Berenguer. I intentionally choose the word *witness* over *supervisor* since Mr. Berenguer only kept me informed of how fast the projects progressed and would share his plans for the upcoming synthetic steps. But there was nothing to discuss really, he managed everything by himself.

After the project associated with the preparation of oseltamivir congeners was completed, Mr. Berenguer initiated a similar project revolving around the chemistry of an oseltamivir bioisoster possessing a phosphonate functional group. As a result, several potent tamifosfor sialomimetics were made but, more importantly, other tamifosfor derivatives were equipped with various linkers to conjugate with oligonucleotides. These compounds were used for developing of the so called DIANA assay. The same neuraminidase inhibitors tethered to biocompatible polymer are now the focus of our research interest since this material could be used in treatment of patients in life threatening conditions. The Thesis does not cover this topic thoroughly since we do not want to disclose our findings right now. But a few details had to be revealed since the Thesis's title contains the noun *theranostics* and therefore a specific targeted therapy based on Berenguer's work is briefly indicated.

Part of the third year of his Ph.D. study Mr. Berenguer spent in Canada, working with Tomas Hudlicky. This internship helped him fine-tune his synthetic skill set. After his return I noticed that he worked totally independently and preferred to report only successfully accomplished work, but not unsolved synthetic problems. My guess is that those very long months in Hudlicky's boot camp gave him a chance to realize that he'd be better off if he sorted out all issues on his own. On top of that, the internship resulted in one paper published in *Tetrahedron Letters*.

Apart from the research associated with chemistry of sialomimetics Mr. Berenguer worked on two different projects that are related to inhibitors of the influenza RNA polymerase. The preparation of a probe against the Cap binding domain of the PB2 subunit required twenty-

one synthetic steps. The other project done by Mr. Berenguer resulted in a detection probe for Alfa screen assay against PA endonuclease, requiring fourteen synthesis steps. These achievements clearly indicate that Mr. Berenguer is capable of accomplishing very challenging tasks.

In the past five years Mr. Berenguer has provided many valuable results that supported our interdisciplinary collaboration with biochemists and virologists. His Thesis is dedicated mainly to the development of tools used in biochemistry and molecular biology. During his Ph.D., three original papers have been published in *Eur. J. Med. Chem*., *Bioorg. Med. Chem. Letters* and in *Tetrahedron Letters*, respectively. One paper has been submitted to *ACS Chemical Biology* this month and the fifth paper is being finalized. Hard copies of both manuscripts are enclosed. Moreover, conjugates of tamifosfor derivatives were described in the DIANA assay patent application as one of the case studies (Example). Please note that the development of DIANA assay is considered at IOCB to be one of the most significant results achieved in the past years.

All detection probes described in the Thesis will be used in the upcoming months in multiple screening campaigns. Therefore, the presented work is very likely going to contribute to the discovery of new inhibitors of neuraminidase, PB2 cap-binding domain, and PA endonuclease.

Mr. Carlos Berenguer has met all the requirements for Ph.D. study and I do recommend his Thesis to enter the defense procedure.

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