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Guest Editorial

Large-Scale Oligonucleotide Synthesis

The use of synthetic oligonucleotides for therapeutic applications represents a new paradigm for rational drug design and discovery. During the past decade, there has been enormous progress in understanding and therapeutic applications of oligonucleotides. Over fifteen oligonucleotides are currently undergoing human clinical trials for the treatment of viral infections (Hepatitis C, CMV-induced retinitis), cancers (non-small cell lung cancer, ovarian cancer, non-Hodgkin's lymphoma, breast cancer) and inflammatory disorders (ulcerative colitis, psoriasis). Very recently both FDA and the EMEA approved Vitravene, the worlds first commercial antisense drug for the treatment of CMV retinitis. Clinical success of antisense drugs has placed increasing emphasis on the development of safe, efficient, and cost-effective processes for oligonucleotide synthesis.

In this issue, we have assembled a series of articles describing the latest research on raw materials, reagents and process chemistries related to large-scale oligonucleotide synthesis. The basic raw materials for oligonucleotide synthesis are nucleosides. Ray and Tang have described an efficient process for multi-kilogram scale synthesis of 2′-*O*-alkyl pyrimidine ribonucleosides, useful for incorporation into second-generation antisense, as well as ribozyme-based constructs. In another nucleoside-related contribution, Appell and Duguid have described an improved oxidation process that eliminates expensive cryogenic reaction conditions. Although the keto nucleoside described in the article is not useful for oligonucleotide-related applications, the general protecting-group strategy is useful for synthesis of other nucleosidic raw materials.

Currently, all oligonucleotides undergoing human clinical trials are made via automated solid-support synthesis utilizing phosphoramidite-coupling chemistry. In general, nucleosides are phosphitylated to produce phosphoramidites. Sanghvi et al. have contributed an article that describes a simplified process for the preparation of nucleosidic phosphoramidites using pyridinium trifluoroacetate (Py·TFA) as a safe and inexpensive activator. Interestingly, the following article by Eleuteri et al. describes the use of Py·TFA as an activator for the oligonucleotide coupling/elongation step. The protocols described in these two articles should eliminate use of 1*H*-tetrazole, a hazardous and expensive reagent currently used in phosphitylation of nucleosides and coupling of phosphoramidites, thus creating a safer process for future manufacture of oligonucleotides. Safety is also a central theme for the next article by Krotz et al. whose contribution describes replacement of a halogenated solvent (CH_2Cl_2) with a non-halogenated alternative (toluene), thereby eliminating the production of large quantities of chlorinated waste in oligonucleotide drug manufacture.

As with any process, raw materials contribute a significant portion to the overall cost of oligonucleotide manufacture. In phosphorothioate oligonucleotide synthesis, the sulfur transfer reagent 3-*H*-1,2-benzodithiol-3-one-1,1-dioxide contributes a large fraction of this cost. The search for cheaper alternatives is therefore an active area of research. Tang et al. report on the use of a five-membered heterocyclic compound as an alternative, and Cheruvallath et al. outline their efforts towards development of a symmetrical disulfide as an efficient and inexpensive sulfur-transfer reagent and their success in decreasing the overall cost of the phosphorothioate oligonucleotide manufacture.

Current solid-phase methodologies produce crude oligonucleotides of remarkably high quality. The crude products, however, must still be subjected to a purification step before they can be considered for clinical use. An article by Deshmukh et al. describes the optimization of several critical parameters required for an anion-exchange based purification method. Such a process optimization lays a firm foundation for a robust very large-scale method in production of therapeutic grade oligonucleotides.

Clearly, solid-support synthesis is a method of choice for oligonucleotide drugs because of its automated protocols and remarkably short synthesis cycle times. However, if a particular oligonucleotide of high purity and short length is required in bulk quantities, synthesis in solution phase is likely to have certain advantages. One such method is high efficiency liquid phase (HELP), described by Bonora et al. This method employs poly(ethylene glycol) as a soluble support for oligonucleotide synthesis, and uses a dimeric synthon approach. The pros and cons of the HELP protocol are discussed along with successful millimolar scale syntheses of 8-mer and 15-mer oligonucleotides.

Large-scale oligonucleotide synthesis is a constantly evolving process. For example, the crude product from the current solid-support synthesis is contaminated with highly homologous failure sequences that arise from incomplete detritylation, coupling, sulfurization, or capping during synthesis. Therefore, chromatographic purification of crude oligonucleotide is necessary at large-scale. Given this scenario, the ability to recover or recycle solvents and reagents, easy manipulation of automated steps, and simplified product isolation would all be very attractive. In this vein, Sebesta et al. have described a new protocol called dimethoxytrityl resin product anchored sequential synthesis (DMT PASS). The preliminary results described in this article are encouraging, and further exploitation of such techniques may lead to useful processes for oligonucleotides.

It is noteworthy that, depending on the structure of the oligonucleotides and reagents such as dimers and trimers

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prepared via solution phase, one can effectively assemble these blocks using a solid-phase approach. With the advances described in these articles, organic process chemists will continue to have options in cheaper raw materials, new reagents, and processes to choose from in their endeavor to synthesize oligonucleotides on large scales. Further oligonucleotide manufacturing processes are likely to integrate a variety of these options, ultimately delivering safe and cost-effective methods for large-scale production of oligonucleotides.

Finally, I would like to acknowledge the contributing authors for their time and effort expended in preparing their manuscripts for this issue. Credit for an up-to-date account of a rapidly moving technology goes to all of the authors. I would also like to thank many expert reviewers who insisted on the inclusion of the latest results and provided their comments in a timely manner.

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Guest Editor

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