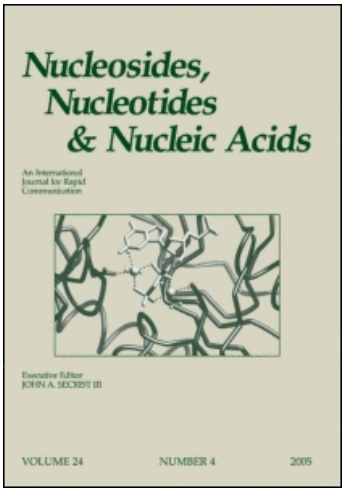


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Efforts Toward Synthesis of Oligonucleotides for Commercialization

Mark Andrade[a]; Anthony S. Scozzari[a]; Douglas L. Cole[a]; Vasulinga T. Ravikumar[a]
[a] Isis Pharmaceuticals, Carlsbad, CA

To cite this Article Andrade, Mark , Scozzari, Anthony S. , Cole, Douglas L. and Ravikumar, Vasulinga T.(1997) 'Efforts Toward Synthesis of Oligonucleotides for Commercialization', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1617 — 1620
To link to this Article: DOI: 10.1080/07328319708006239
URL: http://dx.doi.org/10.1080/07328319708006239

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1617-1620 (1997)

Efforts Toward Synthesis of Oligonucleotides for Commercialization

Mark Andrade, Anthony S. Scozzari, Douglas L. Cole & Vasulinga T. Ravikumar*
Isis Pharmaceuticals,
2292 Faraday Avenue, Carlsbad, CA 92008

Abstract: Recent progress on the development of oligonucleotide phosphorothioates to meet market demands is reported.

1. Introduction: The advent of the "antisense" therapeutic principle has inspired several groups to extend their work beyond the synthesis of modified oligodeoxyribonucleotides to investigation of the potential of small nucleic acid sequences as therapeutic agents. This inspiration is self-evident from the volume of publications reported. A major advantage of the antisense strategy lies in potential specificity of action. In principle, an antisense oligodeoxynucleotide can be designed to target any single gene within the human genome, creating specific therapeutics for any disease for which a causative gene is known. The strategy is proving to be extremely promising.

2. Clinical Candidates: Due to the instability of natural DNA towards nucleases, several structural modifications have been investigated as potential drug classes. Among these, uniformly modified oligodeoxyribonucleotide phosphorothioates have been the first class of compounds to reach the clinic. Several first generation oligonucleotides are in the advanced stages of clinical investigations.

Compound	Sequence	Treatment Status
ISIS 2922	5'-GCGTTTGCTCTTCTTCTTGCG (21-mer)	CMV retinitis Phase III
ISIS 2302	5'-GCCCAAGCTGGCATCCGTCA (20-mer)	ICAM Suppression Phase II
ISIS 3521/ CGP 64128A	5'-GTTCTCGCTGGTGAGTTTCA (20-mer)	PKC-a anti-cancer Phase I
ISIS 5132/ CGP 69846A	5'-TCCCGCCTGTGACATGCATT (20-mer)	c-raf kinase anti-cancer Phase I
ISIS 5320	5'-TTGGGGTT (8-mer)	V3 Loop of gp 120 HIV Pre-clinical
ISIS 2503	5'-TCCGTCATCGCTCCTCAGGG (20-mer)	Ha-ras Pre-clinical

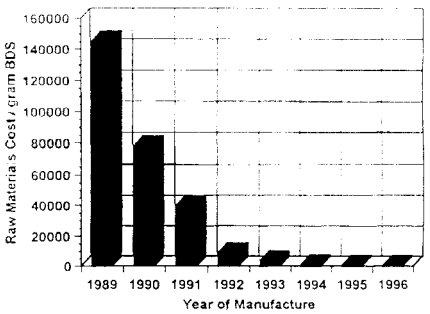
3. Synthesis: Depending on the disease, ultimate demand for specific phosphorothioated oligonucleotide drugs may vary from few hundred grams to several hundred kilograms per year. Hence there is strong need to scale up production of these new drugs in an efficient, cost effective manner. The development of phosphoramidite chemistry and its elaboration into an automated technology have greatly enhanced the ease with which oligonucleotides are synthesized and consequently their availability.

4. Scale: Large scale synthesis of oligonucleotide phosphorothioates has undergone revolutionary changes. Efficient synthesis of 20-mer oligophosphorothioates has been achieved on 80-100 mmole scale (800-1000 g solid support) with only 1.5-fold excess amidite synthons using Pharmacia's OligoProcess synthesizer. Thus several kilograms of bulk drug substance of high purity are being manufactured routinely to support large clinical trials. The second generation OligoProcess will be 10-fold larger, 2 mole scale, equivalent to 5 kg daily purified drug output, or about 1.5 tons annual purified drug output per synthesizer. At this scale, oligonucleotides can be manufactured at multi-ton levels for the market using the same technology.

5. Purification: Currently the crude oligomers are purified by reversed-phase HPLC. We have shown that silica- and polymer-based purifications are equivalent. The loading capacities are high, on the order of 30+ mg/mL of column volume. At 15 kg/run (2 mole) manufacturing scale, 1 1.5m x 0.3m high capacity reversed-phase HPLC column would require one run to purify the crude material ti IND specifications. We have recently developed ion-exchange alternative which completely eliminates use of organic solvents; uses only water and salt.

Cost of raw materials for phosphorothioate oligonucleotide synthesis - a history ...

- ▲ BDS costs per gram thru the 1980's, using "gene machines", were very high
- ▲ By 1991, Isis had lowered raw material costs by 75%
- ▲ In 1992, Isis reduced costs 79% vs. 1991 cost
- ▲ During 1993-1994, Isis process chemists cut BDS costs by 90% vs. 1992 cost
- ▲ Net result: since 1989, Isis has cut BDS cost by 99.4%



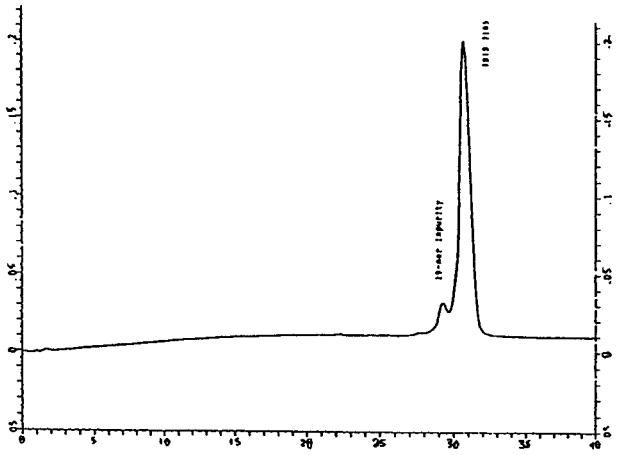


Fig. 1. Analytical SAX HPLC after purification.

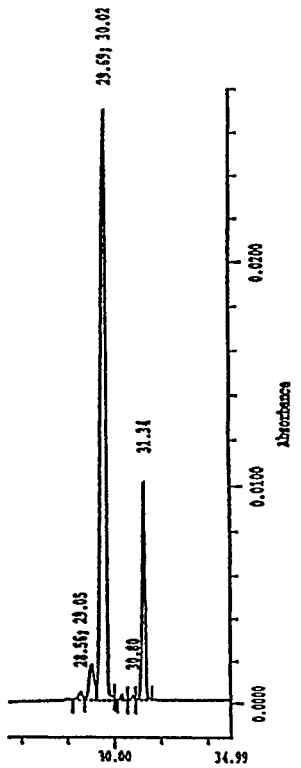


Fig. 2. Capillary gel electrophoresis of the synthesized oligonucleotide. Homothymidine 23-mer was used as internal standard.

6. Characterization: The purified bulk drug substance is rigorously analyzed and the structure confirmed by various means such as UV spectrometry, PAGE, capillary gel electrophoresis, proton, carbon and phosphorus nmr, mass spectrometry-MALDI-TOF or electrospray, base composition analysis and sequencing. Fig. **1** and **2** are CGE and SAX of a typical of bulk drug substance 20-mer phosphorothioate.

7. Cost: The cost of raw materials for phosphorothioate oligonucleotide synthesis has undergone several changes.

The labor cost in R&D were high at small scale, but have been cut 80% since 1992 as scale-up was done to 100 mmole. At ton-scales and 300 day/year operation will require the same operators and less control labor/gram, reducing labor costs to 1% of the cost of the raw materials. Thus assuming systemic phosphorothioate oligomer dosing at 1 mg/kg three times per week, requires 10.5 g/year bulk drug substance per patient. At full-scale manufacturing costs which can be projected based on full success of current development chemistry efforts, the cost to patients per year of novel therapy will be consistent with the cost of other current chronic drug therapies.

In summary, we can state that antisense drugs can be and will be scaled up, at costs supporting very effective patient therapy at a fair profit.

References:

1. Crooke, S. T. In *Burger's Medicinal Chemistry and Drug Discovery*; M. E. Wolff, Ed.; John Wiley & Sons: New York, **1995**; Vol. 1; pp 863.
2. Ravikumar, V. T.; Andrade, M.; Wyrzykiewicz, T. K.; Scozzari, A.; Cole, D. L. *Nucleosides Nucleotides*, **1995**, *14*, 1219.
3. D. L. Cole, *XII International Round Table: Nucleosides, Nucleotides and Their Applications*, La Jolla, September 15-19, **1996**.