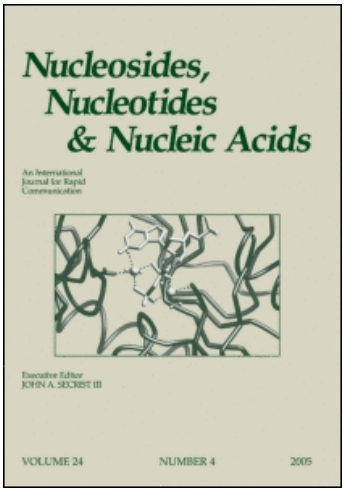

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

MULTIPLE OLIGONUCLEOTIDE SYNTHESIS IN TANDEM ON SOLID-PHASE SUPPORTS FOR SMALL AND LARGE SCALE SYNTHESIS

R. T. Pon[a]; S. Yu[a]; Y. S. Sanghvi[b]
[a] Dept. Biochem. & Molec. Biol., University of Calgary, Calgary, Canada [b] Isis Pharmaceuticals, Carlsbad, California, U.S.A.

Online publication date: 31 March 2001

To cite this Article Pon, R. T. , Yu, S. and Sanghvi, Y. S.(2001) 'MULTIPLE OLIGONUCLEOTIDE SYNTHESIS IN TANDEM ON SOLID-PHASE SUPPORTS FOR SMALL AND LARGE SCALE SYNTHESIS', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 985 — 989
To link to this Article: DOI: 10.1081/NCN-100002474
URL: http://dx.doi.org/10.1081/NCN-100002474

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 985–989 (2001)

MULTIPLE OLIGONUCLEOTIDE SYNTHESIS IN TANDEM ON SOLID-PHASE SUPPORTS FOR SMALL AND LARGE SCALE SYNTHESIS

R. T. Pon,[1,*] S. Yu,[1] and Y. S. Sanghvi [2]

[1]Dept. Biochem. & Molec. Biol., University of Calgary,
Calgary, AB, Canada T2N 4N1
[2]Isis Pharmaceuticals, 2292 Faraday Ave., Carlsbad, California 92008

ABSTRACT

Multiple oligonucleotides linked end-to-end in tandem can be synthesized by adding a nucleoside to the 5′-OH end of a prior sequence. Nucleosides with 3′-succinyl or *Q-Linker* arms are coupled with HBTU/DMAP. Alternatively, new phosphoramidite reagents with 3′-ester linkages can be used. Hydroxyl or amino supports can also be used as universal starting materials. Treatment with NH_4OH cleaves the 3′-ester to yield only 3′-OH groups and no unwanted 3′-phosphorylated products occur.

Increasing demand for oligonucleotides on both small and large scales requires new methods which are faster or cheaper. Synthesis of oligonucleotides linked end-to-end on the same solid-phase support (tandem synthesis) can be used to prepare sets of oligonucleotides, such as PCR primers, forward & reverse sequencing primers, multiplexed genotyping primers, and double-stranded fragments, which are used together. This technique reduces labor by reducing the number of times synthesizers must be set-up and allowing multiple products to be handled as single sets. In large scale production, tandem synthesis of multiple copies of the same sequence increases the amount of product obtained from each synthesis column. Since the support is the most expensive single material, this can significantly reduce the cost of large scale synthesis.

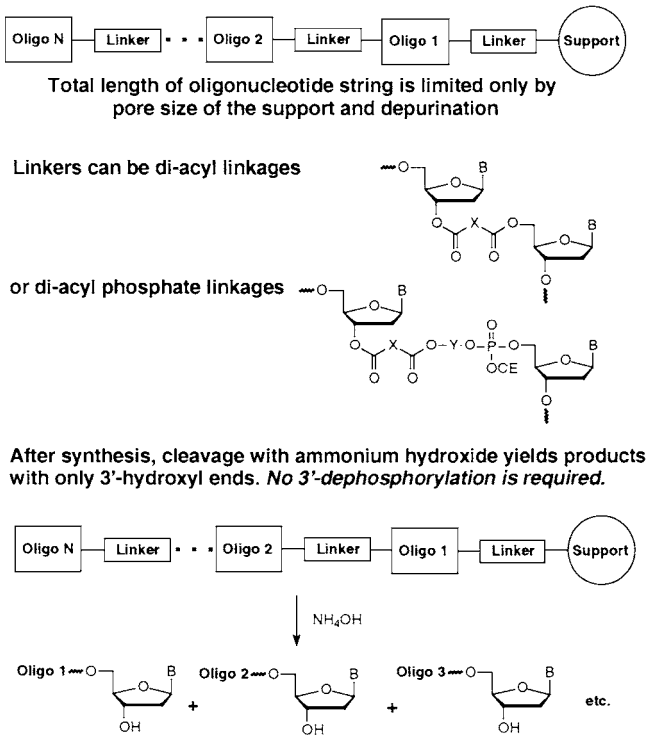

Figure 1. Tandem oligonucleotides synthesis.

Originally, a phosphoramidite linking reagent (1) was described for tandem synthesis, but we have modified this approach by employing linking reagents with cleavable 3′-ester linkages (Fig. 1). These reagents are either nucleoside-3′-carboxylates (Fig. 2a) or new linker-phosphoramidite (Fig. 2b) reagents. Automated coupling is performed by using either HBTU/DMAP (2,3) (nucleoside-3′-carboxylates) or conventional phosphoramidite chemistry. These reagents work equally well on either underivatized hydroxyl or amino supports (which act as "Universal" supports) or 5′-OH groups. In the latter case, the terminal 5′-OH position of a prior sequence serves as the starting point for the next synthesis to allow multiple tandem sequences. Upon cleavage with NH_4OH, the ester linkage to the 3′-position is hydrolyzed and no 3′-phosphorylated products are produced. Use of the *Q-Linker* arm (4) instead of succinic acid also decreases the time required for cleavage from the support from ~60–90 min to only 2 min (phosphodiester backbones) or 10 min (phosphorothioate backbones).

Tandem synthesis was performed on an ABI 394 DNA synthesizer. An appropriately protected nucleoside with a 3′-*O*-hydroquinone diacetyl hemiester (0.15 mmol) and diisopropylethylamine (0.15 mmol) were dissolved in acetonitrile (1 ml) and installed on a spare base position. HBTU (0.15 mmol) and DMAP (0.15 mmol) were dissolved in acetonitrile (1 ml) and installed on another spare base position.

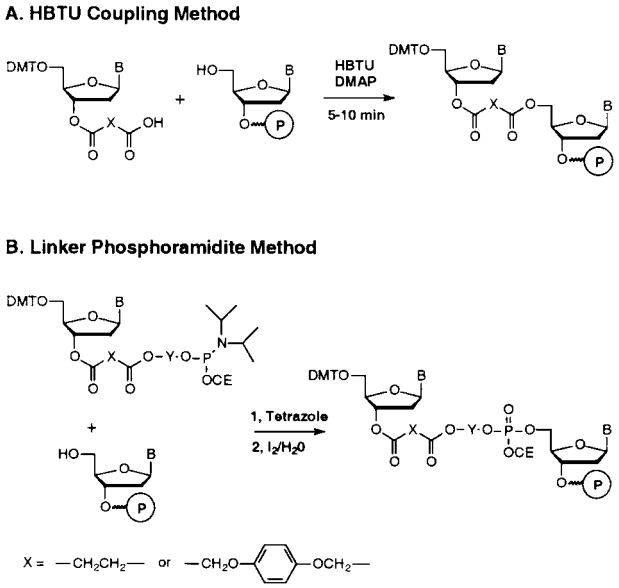

Figure 2. Nucleoside coupling strategies.

Underivatized long chain alkylamine or glycerol derivatized CPG (∼10 mg) was used. A custom begin procedure was created which simultaneously delivered both the nucleoside and HBTU/DMAP reagents to the column. After a 5 min wait step, the column was capped with Cap A and B reagents and the first oligonucleotide sequence was performed using an unmodified synthesis cycle. After completion (trityl-off mode), no cleavage step was performed. The synthesis column was left in place, the synthesizer was programmed with a second sequence, and the synthesizer was restarted as above to run the custom begin procedure and perform the second synthesis. This procedure can be repeated until the total length of the tandem oligonucleotides exceeds the pore volume of the support, i.e. ∼40–50 bases for 500Å or ∼150 bases for 1000Å CPG. When the desired sequences are complete, a short ammonium hydroxide (2 min) cleavage simultaneously releases all of the products from the support. Removal of N-protecting groups is then performed (55°, 16 h) as usual.

The HBTU/DMAP coupling reagent can also be eliminated if the nucleoside-3′-*O*-hemiesters (succinyl or *Q-Linker*) are converted into phosphoramidite reagents. This is done by addition of a diol, such as ethylene glycol, to convert the hemiester into a diester. The resulting hydroxyl terminated compound is then converted into a phosphoramidite using 2-cyanoethyl diisopropylchlorophosphoramidite. In this case, the linker phosphoramidite is added using a conventional phosphoramidite coupling cycle. This reagent can be used with underivatized aminoalkyl or glycerol CPG, which again serve as "Universal" supports. After the first base is added via the appropriate linker phosphoramidite, the remainder of

the sequence is made by conventional means. Treatment with NH_4OH releases the oligonucleotide from the support with a free 3′-OH terminus and the phosphate-modified support is discarded.

The linker phosphoramidite reagents can also by used to create tandem linked sequences by coupling to the terminal 5′-hydroxyl position of a prior sequence. After cleavage, products will have 3′-OH termini, but a 5′-hydroxyalkylphosphate group will remain from each linker phosphoramidite. However, 5′-hydroxyalkylphosphate modification is not expected to interfere in the oligonucleotides ability to act as a primer.

We have prepared tandem strings of dA_6, dA_{10}, and dA_{14} sequences; 24 base-long primers in either duplicate, triplicate, or quadruplicate (total of 96 bases); and two complementary 20-mers, which on cleavage spontaneously formed a dsDNA fragment. In the first case, three products were obtained in a single mixture with the expected electrophoretic mobilities when checked by either capillary electrophoresis (CE) or denaturing polyacrylamide gel electrophoresis (PAGE). In the second case, CE analysis confirmed that each syntheses produced the same product as a single control synthesis. Finally, in the last example, electrophoresis under non-denaturing conditions confirmed the presence of a single double-stranded product.

Selective cleavage of two different sequences can also be obtained by using a succinyl linker for the first sequence and then a *Q-linker* (4) arm for the second. An short (2 min) NH_4OH treatment released the oligonucleotide from the *Q-Linker* while a second longer (60 min) treatment cleaved the succinyl linker. CE analysis showed only 2.3% cross-contamination of the first product released and no detectable cross-contamination of the last product released. This was sufficient purity for most purposes and was comparable to the amount of N-1 failure product present in many crude synthesis products.

Multiple copies of the same sequence can also be made to increase the amount of product obtained from a single column. This will be useful to prepare large quantities of either full-length pharmaceutically useful sequences or smaller intermediates for eventual "block" couplings. In one example, a dA_6 oligonucleotide was synthesized once (as control) and then three times in tandem. The single control yielded 1,580 A_{260} units of product per gram of support which was 85% pure (by CE analysis), while the tandem synthesis yielded 5,460 A_{260} units of product per gram of support (3.5 times as much as the single synthesis). This was also slightly purer (92%), presumably because coupling efficiency increases as the oligonucleotide string gets longer. In another example, the 5-mer dCGGTA which causes apoptosis (5) was made ten times in tandem. This gave 8.2 times as much material as obtained from a single synthesis. Tandem synthesis can also be combined with support recycling (6) and we have combined double tandem synthesis with five consecutive uses of the same support to obtain ten copies of an ISIS 2302 20-mer phosphorothioate from a single synthesis column. Therefore, considerable savings in the amount of solid-phase support consumed in large scale production is possible.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

REFERENCES

1. Hardy, P.M.; Holland, D.; Scott, S.; Garman, A.J.; Newton, C.R.; McLean, M.J. *Nucl. Acids Res*., **1994**, *22*, 2998–3004.
2. Pon, R.T.; Yu, S.; Sanghvi, Y.S. *Bioconjugate Chem*., **1999**, *10*, 1051–1057.
3. Pon, R.T.; Yu, S. *SynLett*, **1999**, 1778–1780.
4. Pon, R.T.; Yu, S. *Nucl. Acids Res*., **1997**, *25*, 3629–3635.
5. Tidd, D.M.; Spiller, D.G.; Broughton, C.M.; Norbury, L.C.; Clark, R.E.; Giles, R.V. *Nucl. Acids Res*., **2000**, *28*, 2242–2250.
6. Pon, R.T.; Yu, S.; Guo, Z.; Sanghvi, Y.S. *Nucl. Acids Res*., **1999**, *27*, 1531–1538.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!