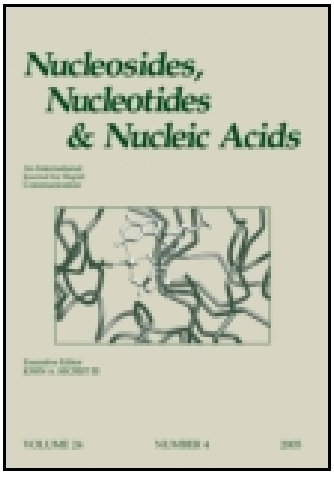


Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

A New Approach to Oligonucleotide Synthesis in Solution

Colin B. Reese [a] & Quanlai. Song [a]
[a] Department of Chemistry , King's College London , Strand, London , WC2R 2LS, UK
Published online: 22 Aug 2006.

To cite this article: Colin B. Reese & Quanlai. Song (1998) A New Approach to Oligonucleotide Synthesis in Solution, Nucleosides and Nucleotides, 17:9-11, 2027-2031, DOI: 10.1080/07328319808004743

To link to this article: http://dx.doi.org/10.1080/07328319808004743

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 2027-2031 (1998)

A NEW APPROACH TO OLIGONUCLEOTIDE SYNTHESIS IN SOLUTION

Colin B. Reese* and Quanlai Song

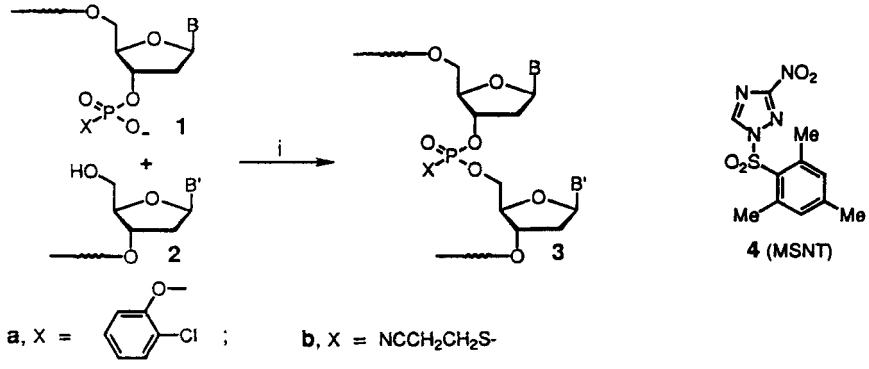
Department of Chemistry, King's College London,
Strand, London WC2R 2LS, UK

ABSTRACT: A new approach, based on the use of 3'-*H*-phosphonate building blocks, is described for the synthesis of oligonucleotides and their phosphorothioate analogues in solution.

The phosphotriester[1], phosphoramidite[2] and *H*-phosphonate[3] approaches have all proved to be effective for the chemical synthesis of oligonucleotides. The phosphotriester approach has been used most widely in solution phase synthesis while the phosphoramidite and *H*-phosphonate approaches have been used almost exclusively in solid phase synthesis. The possibility of oligonucleotides and their phosphorothioate analogues being used as chemotherapeutic agents[4] has made their large scale synthesis a matter of much importance. So far, the demand for relatively large quantities of these materials has been met by the scaling-up of solid phase synthesis. However, we believe that if a specific oligonucleotide or oligonucleotide phosphorothioate sequence becomes licensed as a drug and really large (say, multikilogram) quantities of it are required, solution phase synthesis is likely to become the method of choice.

Perhaps the most widely used strategy for the synthesis of oligodeoxyribonucleotides in solution involves (SCHEME 1) a coupling reaction between a protected nucleoside or oligonucleotide 3'-(2-chlorophenyl) phosphate[5] **1a** and a protected nucleoside or oligonucleotide **2** with a free 5'-hydroxy function to give a phosphotriester **3a**. A coupling agent such as 1-(mesitylene-2-sulfonyl)-3-nitro-1-*H*-1,2,4-triazole (MSNT)[6] **4** is required. This strategy has also been used in the synthesis of phosphorothioate analogues of oligonucleotides. Coupling is then effected in the same way between a protected nucleoside or oligonucleotide 3'-*S*-(2-cyanoethyl) phosphorothioate[7,8] **1b** and component **2**. The main disadvantage of this strategy is that some concomitant 5'-sulfonation (usually $< 5\%$) of the second component **2** occurs[9]. This leads to lower coupling yields and also complicates the purification process. We therefore set out to devise

*Tel no. : +44171 - 873 2260; fax no. : +44171 - 873 2899

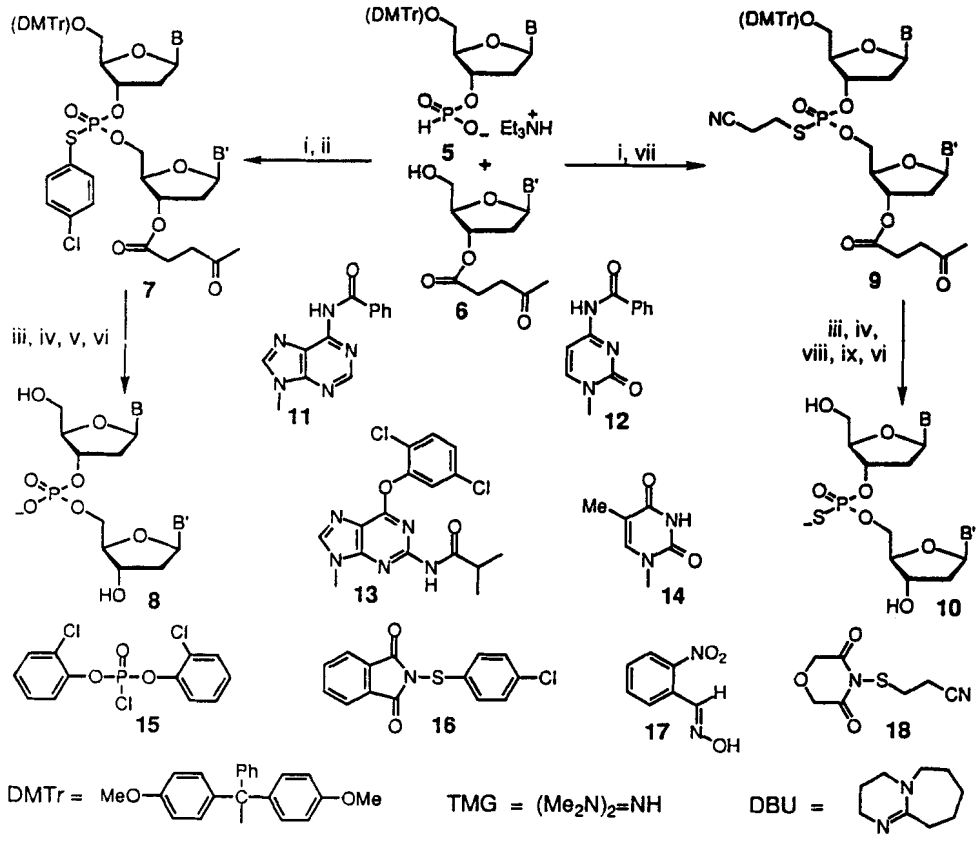


SCHEME 1 *Reagents and conditions*: i, MSNT **4**, C_5H_5N, room temp., 30 min.

a new procedure for the synthesis of oligonucleotides in solution that (a) does not involve side-reactions and (b) is equally suitable for the preparation of oligonucleotides and their phosphorothioate analogues.

The monomeric building blocks required in the new procedure are triethylammonium 5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyribonucleoside 3'-*H*-phosphonates[10] **5** (B = **11** - **14**). When triethylammonium 6-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine 3'-*H*-phosphonate (DMTr-*A*p(H))† **5** (B = **11**) (1.2 mol. equiv.), 4-*N*-benzoyl-3'-*O*-levulinyl-2'-deoxycytidine (HO-*C*-Lev) **6** (B = **12**) (1.0 mol. equiv.) and di-(2-chlorophenyl) phosphorochloridate **15** (1.5 mol. equiv.) were allowed to react together in pyridine - dichloromethane at -40°C, the corresponding fully-protected dinucleoside *H*-phosphonate was obtained apparently in quantitative yield within 5-10 min. No attempt was made to isolate the latter intermediate, but *N*-[(4-chlorophenyl)sulfanyl]phthalimide[11] **16** (2.0 mol. equiv.) was added to the reactants which were maintained at -40°C. After 15 min, the products were worked up and chromatographed to give DMTr-*Ap(s')C*-Lev† **7** (B = **11**, B' = **12**) in *ca.* 99% isolated yield. Thus, both the coupling and the sulfur-transfer steps proceeded relatively quickly and virtually quantitatively at -40°C. The fully-protected dinucleoside phosphorothioate **7** (B = **11**, B' = **12**) was converted into *extremely pure* d[ApC] **8** (B = adenin-9-yl, B' = cytosin-1-yl) by a four step procedure (SCHEME 2, steps iii-vi). Detritylation (step iii) was carried out at -50°C under conditions under

†In this system of abbreviations for protected oligonucleotides, nucleoside residues and internucleotide linkages are italicized if they are protected in some defined way. In the present context *A*, *C*, *G* and T represent 2'-deoxyadenosine protected on *N*-6 with a benzoyl group (as in **11**), 2'-deoxycytidine protected on *N*-4 with a benzoyl group (as in **12**), 2'-deoxyguanosine protected on *N*-2 and on *O*-6 with isobutyryl and 2,5-dichlorophenyl groups (as in **13**) and unprotected thymidine, respectively; *p(s)* and *p(s')* represent *S*-(2-cyanoethyl) and *S*-(4-chlorophenyl) phosphorothioates, respectively, and p(H) which is not protected and therefore not italicized represents an *H*-phosphonate monoester if it is attached to a monomer (as in **5**) or placed at the end of a sequence.



SCHEME 2 *Reagents and conditions*: i, **15**, C_5H_5N, CH_2Cl_2, -40°C, 5-10 min; ii, a, **16**, C_5H_5N, CH_2Cl_2, -40°C, 15 min, b, C_5H_5N - H_2O (1:1 v/v), -40°C to room temp.; iii, 4 M HCl / dioxane, CH_2Cl_2, -50°C, 5 min; iv, Ac_2O, C_5H_5N, room temp., 15 h; v, **17**, TMG, MeCN, room temp., 12 h; vi, a, conc. aq. NH_3 (*d* 0.88), 50°C,15 h, b, Amberlite IR-120 (plus), Na^+ form, H_2O; vii, a, **18**, C_5H_5N, CH_2Cl_2, -40°C, 15 min, b, C_5H_5N - H_2O (1:1 v/v), -40°C to room temp.; viii, DBU, Me_3SiCl, CH_2Cl_2, room temp., 30 min; ix, **17**, DBU, MeCN, room temp., 12 h.

which no concomitant depurination occurred. As other workers[12,13] had previously demonstrated with related *S*-aryl phosphorothioates, unblocking of the internucleotide linkage with oximate ions[6,14] (step v) leads exclusively to cleavage of the phosphorus-sulfur bond.

The protocol for the preparation of oligonucleotide phosphorothioates differs from that used for oligonucleotides only in that sulfur-transfer is effected with 4-[(2-cyanoethyl)-sulfanyl]morpholine-3,5-dione[15] **18** rather than with *N*-[(4-chlorophenyl)sulfanyl]phthalimide **16**. Triethylammonium 6-*O*-(2,5-dichlorophenyl)-5'-*O*-(4,4'-dimethoxytrityl)-2-*N*-isobutyryl-2'-deoxyguanosine 3'-*H*-phosphonate (DMTr-*G*p(H)) **5** (B = **13**) (1.2 mol. equiv.), 6-*N*-benzoyl-3'-*O*-levulinyl-2'-deoxyadenosine (HO-*A*-Lev) **6** (B' = **11**) (1.0 mol. equiv.) and di-(2-

chlorophenyl) phosphorochloridate **15** (1.5 mol. equiv.) were allowed to react together in pyridine - dichloromethane solution at -40°C for 5-10 min (SCHEME 2, step i). 4-[(2-Cyanoethyl)sulfanyl]morpholine-3,5-dione **18** (2.0 mol. equiv.) (step vii) was then added while the reactants were maintained at -40°C. After 15 min, the products were worked up and fractionated by chromatography on silica gel to give DMTr-*Gp(s)A*-Lev **9** (B = **13**, B' = **11**) in *ca.* 99% isolated yield. This material was unblocked by a five step procedure (SCHEME 2, steps iii, iv, viii, ix and vi). The *S*-(2-cyanoethyl) protecting group was removed from the internucleotide linkage by treatment (step viii) with DBU[8] in dichloromethane under strictly anhydrous conditions, and the 6-*O*-(2,5-dichlorophenyl) protecting group was removed from the guanine residue by oximate treatment[16] (step ix). Extremely pure d[Gp(s)A] **10** (B = guanin-9-yl, B' = adenin-9-yl) was obtained after the final ammonolysis step (step vi).

This new approach to the solution phase synthesis of oligonucleotides and oligonucleotide phosphorothioates lends itself both to stepwise (i.e. the addition of one nucleotide residue at a time) and to block (i.e. the addition of two or more nucleotide residues at a time) coupling reactions and, like other solution phase processes, it has the potential to be scaled-up almost without limit. The tetranucleoside triphosphate d[TpGpApC], the tetranucleoside triphosphorothioate d[Cp(s)Tp(s)Gp(s)A] and the chimeric tetranucleoside diphosphate phosphorothioate d[TpGp(s)ApC] were all prepared by stepwise coupling and in high overall yields. The octanucleoside heptaphosphorothioate d[Tp(s)Tp(s)Gp(s)Gp(s)Gp(s)Gp(s)Tp(s)T] was prepared by coupling together the two tetramer blocks Ac-T*p(s)*T*p(s)Gp(s)Gp*(H) and HO-*Gp(s)Gp(s)*T*p(s)*T-Bz to give Ac-T*p(s)*T*p(s)Gp(s)Gp(s)Gp(s)Gp(s)*T*p(s)*T-Bz which was isolated in 91% yield and then unblocked in three steps (steps viii, ix and vi in SCHEME 2). We believe that this new approach to solution phase synthesis will become the method of choice for the large scale preparation at least of oligonucleotides and oligonucleotide phosphorothioates of moderate molecular weight.

REFERENCES

1. Reese, C. B. *Tetrahedron*, **1978**, *34*, 3143-3179.
2. Beaucage, S. L. in *Methods in Molecular Biology, Vol. 20, Protocols for Oligonucleotides and Analogs*, Agrawal, S., Ed., Humana Press, Totowa, 1993, pp 33-61.
3. Froehler, B. C. in *Methods in Molecular Biology, Vol. 20, Protocols for Oligonucleotides and Analogs*, Agrawal, S., Ed., Humana Press, Totowa, 1993, pp 63-80.
4. Gura, T. *Science*, **1995**, *270*, 575-577.
5. Chattopadhyaya, J. B.; Reese, C. B. *Nucleic Acids Res.*, **1980**, *8*, 2039-2054.
6. Reese, C. B.; Titmus, R. C.; Yau, L. *Tetrahedron Lett.*, **1978**, 2727-2730.
7. Liu, X.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1*, **1995**, 1685-1695.
8. Reese, C. B.; Song, Q.; Rao, M. V.; Beckett, I. *Nucleosides Nucleotides*, in press.
9. Reese, C. B.; Zhang, P.-Z. *J. Chem. Soc., Perkin Trans. 1*, **1993**, 2291-2301.
10. Ozola, V.; Reese, C. B.; Song, Q. *Tetrahedron Lett.*, **1996**, *37*, 8621-8624.
11. Behforouz, M.; Kerwood, J. E. *J. Org. Chem.*, **1969**, *34*, 51-55.
12. Kamimura, T.; Tsuchiya, M.; Urakami, K.; Koura, K.; Sekine, M.; Shinozaki, K.; Muira, K.; Hata, T. *J. Am. Chem. Soc.*, **1984**, *106*, 4552-4557.

13. Wreesman, C. T. J.; Fidder, A.; Veeneman, G. H.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.*, **1985**, *26*, 933-936.
14. Reese, C. B.; Zard, L. *Nucleic Acids Res.*, **1981**, *9*, 4611-4626.
15. Klose, J.; Reese, C. B.; Song, Q. *Tetrahedron*, in press.
16. Reese, C. B.; Skone, P. A. *J. Chem. Soc., Perkin Trans. 1*, **1984**, 1263-1271.