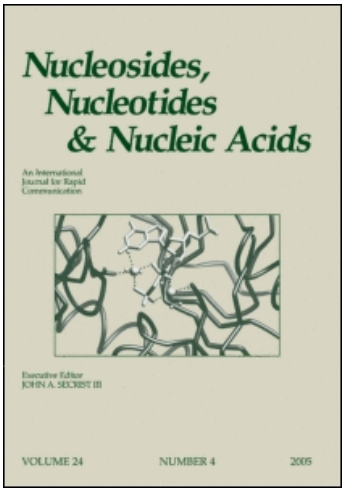

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A NEW "NATIVE LIGATION" PROCEDURE FOR PEPTIDE-OLIGONUCLEOTIDE CONJUGATION

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A NEW "NATIVE LIGATION" PROCEDURE FOR PEPTIDE-OLIGONUCLEOTIDE CONJUGATION

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ABSTRACT

"Native ligation", a powerful method of joining peptide fragments, has been applied successfully to peptide-oligonucleotide conjugation. Novel reagents are described for the solid-phase synthesis of peptide *N*-terminal thioesters and 5′-cysteinyl oligonucleotides suitable for ligation reactions.

Oligonucleotides and their analogues are used widely as sequence-specific reagents to block gene expression within cells (1–2). Yet oligonucleotide efficacy in cell culture is often limited by poor cellular uptake. Among the many molecules that have been reported to enhance cell delivery of oligonucleotides are a number of peptide sequences and several peptide-oligonucleotide conjugates have been reported to show enhanced cell penetration (3–5). However, systematic studies for good cell accessibility and compartmentalisation as well as correlation between structure and biological activity are still lacking for peptide-oligonucleotide conjugates. This is because such studies have been hampered by the often cumbersome and inefficient methods required for their chemical synthesis.

Our ongoing project (6), is to explore new approaches for the chemical synthesis of peptide-oligonucleotide conjugates that are efficient and convenient for use with conventional solid-phase synthetic procedures and with the minimum of post-assembly manipulation. The methods must be suitable for a wide range

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of oligonucleotides and their analogues and for peptides of type and length required for cell uptake studies. To this end, we have developed a novel strategy for conjugation of peptides to oligonucleotides based on "native ligation" technology (7–8) used previously in total protein synthesis to generate an amide bond by reaction of a thioester with a cysteine moiety containing free amino and thiol groups. Peptide *C*-terminal thioesters are available mainly by Boc/benzyl chemistry, but a few new methods have been published recently that make use of milder Fmoc/*tert*-butyl methodology (9–11). Our route involves joining of an *N*-terminal thioester-functionalized peptide to a 5′-cysteinyl oligonucleotide (12).

New reagents were required to accomplish the task. Pentafluorophenyl *S*-benzyl thiosuccinate was used in the final coupling step in standard Fmoc-based solid-phase peptide assembly (Fig. 1). Alternatively, the corresponding *S*-benzyl

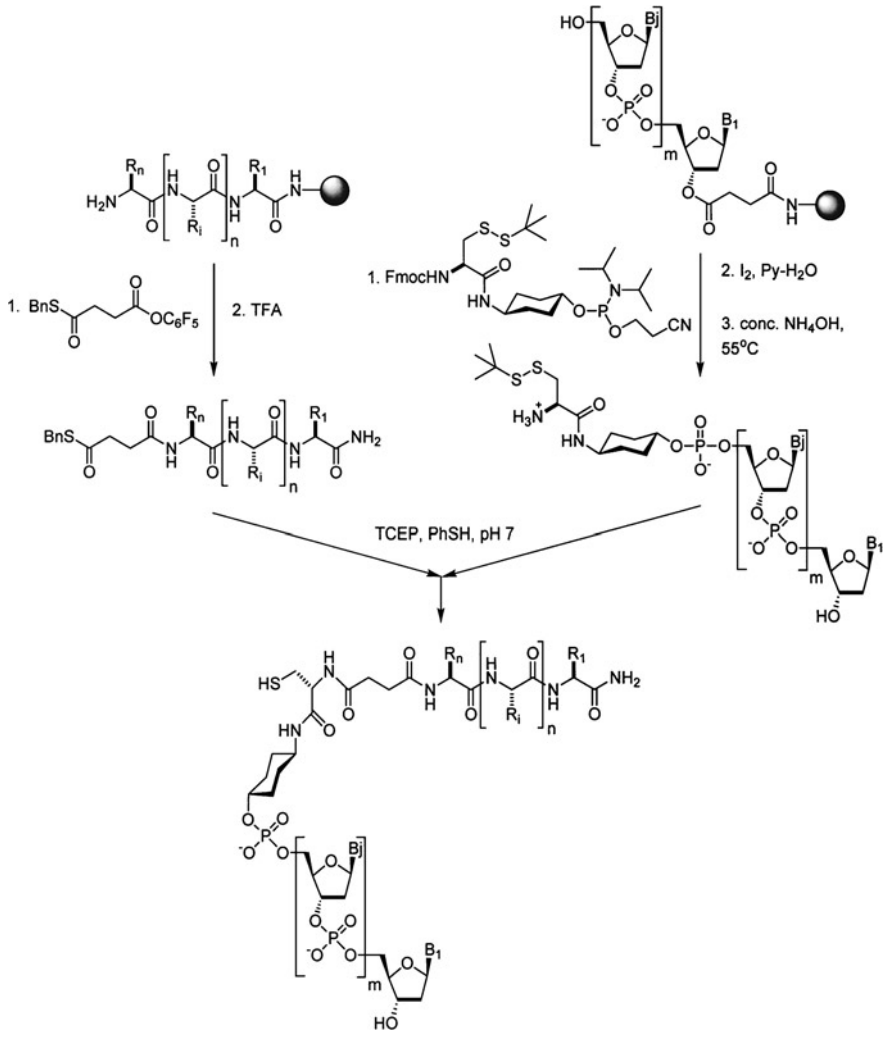

Figure 1. Scheme of peptide-oligonucleotide conjugation by "native ligation".

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thiosuccinic acid can be used in conjunction with a suitable coupling reagent (e.g. PyBOP or HATU). TFA deprotection generated in solution peptides bearing an *N*-terminal *S*-benzyl thioester functionality, but care must be taken to use benzyl mercaptan in place of common thiol scavengers to preserve the integrity of the thioester moiety. *S*-Benzyl thioesters were shown to withstand 90% TFA deprotection unchanged and to be sufficiently stable to be stored in frozen 0.1% aqueous TFA solution for several months.

The second reagent family, *N*-α-Fmoc-*S*-(*tert*-butylsulfenyl or trityl) protected- L-cysteinyl aminoalkyl 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidites, were used in the final coupling step in standard phosphoramidite solid-phase oligonucleotide assembly (Fig. 1) (12–13). The aminoalkyl moiety is derived from a secondary alcohol such as 4-aminocyclohexanol. Deprotection with concentrated aqueous ammonia solution at 55°C generated 5′-*S*-protected-L-cysteinyl functionalized oligonucleotides. The Fmoc group can be removed independently by treatment with 20% piperidine in DMF prior to total deprotection. Functionalized peptides and oligonucleotides assembled in high yield were used without purification in native ligation reactions in aqueous/organic mixture using the water-soluble reducing agent *tris*-(2-carboxyethyl)phosphine to remove the *tert*-butylsulfenyl *S*-protecting group *in situ* and thiophenol as a conjugation enhancer. Prior $AgNO_3$ treatment followed by DTT precipitation was needed to remove trityl *S*-protection from respective oligonucleotides. A range of conjugates was prepared by this route and purified by reversed phase HPLC and their respective molecular masses confirmed by MALDI-TOF mass spectrometry (12).

We are now extending the conjugation chemistry to phosphorothioates and 2′-*O*-alkyl oligoribonucleotides that are more commonly used for antisense experiments. In applications of such conjugates in cellular uptake studies, the presence of a free thiol in the cysteine residue remaining at the joint between peptide and oligonucleotide parts might be useful for attachment of a fluorescent or other reporter group.

REFERENCES

1. Cook, P. D. *Nucleosides & Nucleotides*, **1999**, *18*, 1141–1162.
2. Chadwick, D. J.; Cardew, G. *Oligonucleotides as Therapeutic Agents*, **1997**, John Wiley, Chichester, UK.
3. Bongartz, J.-P.; Aubertin A.-M.; Milhaud P. G.; Lebleu, B. *Nucleic Acids Res*., **1994**, *22*, 4681–4688.
4. Allinquant, B.; Hantraye, P.; Mailleux, P.; Moya, K.; Bouillot, C.; Prochiantz, A. J. *Cell Biol*., **1995**, *128*, 919–927.
5. Astriab-Fisher, A.; Sergueev, D. S.; Fisher, M.; Ramsay Shaw, B.; Juliano, R. L. *Biochem. Pharmacol*., **2000**, *60*, 83–90.
6. Peyrottes, S.; Mestre, B.; Burlina, F.; Gait, M. J. *Tetrahedron*, **1998**, *54*, 12513–12522.
7. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science*, **1994**, *266*, 776–779.

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8. Muir, T. W.; Dawson, P. E.; Kent, S. B. H. *Methods Enzymol*., **1997**, *289*, 266–298.
9. Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc*., **1999**, *121*, 11369–11374.
10. Shin, Y.; Winans, K.; Backes, B.; Kent, S. B. H.; Ellmann, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc*., **1999**, *121*, 11684–11689.
11. Swinnen, D.; Hilvert, D. *Org. Letters*, **2000**, *2*, 2439–2442.
12. Stetsenko, D. A.; Gait, M. J. *J. Org. Chem*., **2000**, *65*, 4900–4908.
13. Stetsenko, D. A.; Gait, M. J. *Nucleosides*, *Nucleotides & Nucleic Acids*, **2000**, in press.

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