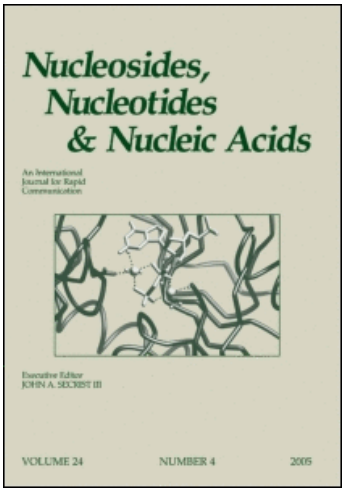


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Studies on Alternate Strand Triple Helix Formation by Oligodeoxyribonucleotides Containing A 3'-3' Phosphodiester Bond

L. De Napoli[a]; A. Galeone[b]; L. Mayol[b]; A. Messere[a]; D. Montesarchio[a]; A. Pepe[b]; G. Piccialli[a]
[a] Dipartimento di Chimica Organica e Biologica, Universita di Napoli "Federico II", Napoli, Italy [b] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Napoli, Italy

To cite this Article De Napoli, L. , Galeone, A. , Mayol, L. , Messere, A. , Montesarchio, D. , Pepe, A. and Piccialli, G.(1998) 'Studies on Alternate Strand Triple Helix Formation by Oligodeoxyribonucleotides Containing A 3'-3' Phosphodiester Bond', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1709 — 1716
To link to this Article: DOI: 10.1080/07328319808004705
URL: http://dx.doi.org/10.1080/07328319808004705

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1709-1716 (1998)

STUDIES ON ALTERNATE STRAND TRIPLE HELIX FORMATION BY OLIGODEOXYRIBONUCLEOTIDES CONTAINING A 3'-3' PHOSPHODIESTER BOND

L.De Napoli[a], A.Galeone[b], L.Mayol[b], A.Messere[a], D.Montesarchio[a], A.Pepe[b] and G.Piccialli[a*]

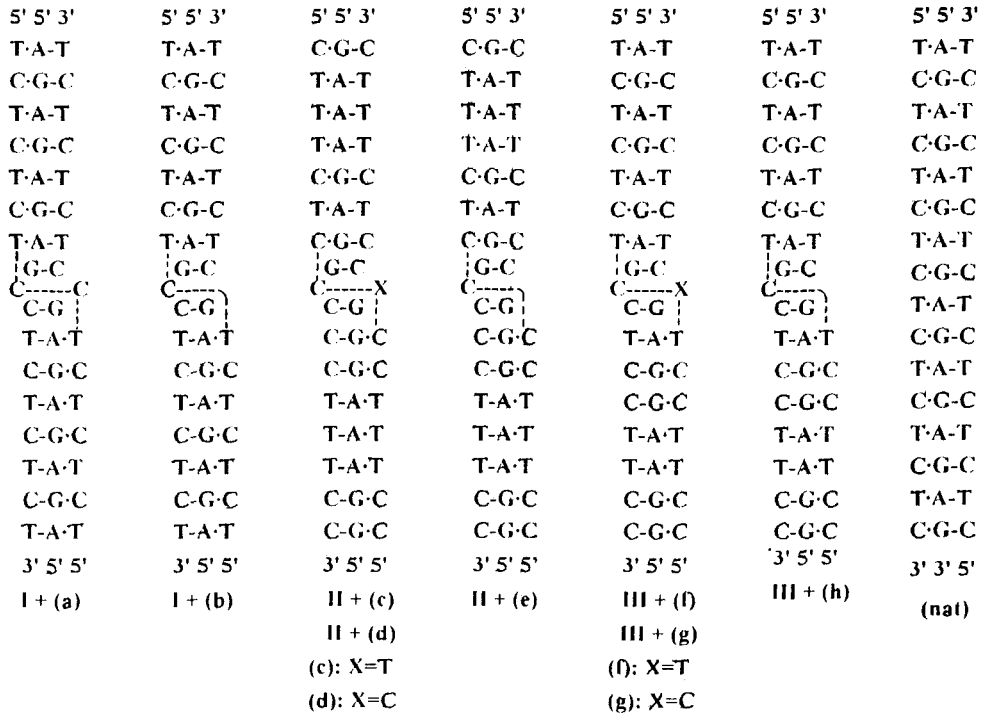
[a]Dipartimento di Chimica Organica e Biologica, Università di Napoli "Federico II", Napoli, Italy;
[b]Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Napoli, Italy.

Abstract. ODNs containing a 3'-3' phosphodiester linkage as inversion of polarity motif have been shown to cooperatively bind to 5'-(purine)$_m$(pyrimidine)$_n$-3' type duplexes by specific alternate strand recognition of the adjacent oligopurine domains. An NMR study has been undertaken to investigate the role of the 3'-3' linked nucleosides and their nearest neighbours in the stabilization of the triple helical complexes.

Efforts towards a wider applicability of synthetic oligodeoxyribonucleotides (ODNs) in the "antigene" approach[1], aimed at sequence-specifically recognizing biologically relevant duplex fragments, include the use of ODNs having a 3'-3' or 5'-5' internucleoside junction able to form triplex structures targeting 5'-(purine)$_m$(pyrimidine)$_n$-3' type duplexes. Several ODN analogues[2], with an appropriate linker connecting the 3'-termini of the two 3'-5' tracts, have been shown to be able to simultaneously and cooperatively bind to the adjacent purine domains on alternate strands of the Watson-Crick duplex, switching strand at the junction between the oligopurine and the oligopyrimidine domains by crossing the major groove.

Recently we[3] have focused our attention on ODNs containing a 3'-3' internucleoside phosphodiester linkage as inversion of polarity motif, proposing a new method for their synthesis and testing their ability at hybridizing complementary duplex fragments. Such molecules were efficiently prepared by a synthetic route which, in alternative to the use of

TABLE



Tm values (°C)/pH for sequences **a-h** and **nat**.

Sequences	a	b	c	d	e	f	g	h	nat
pH = 5.5	44.2	31.0	23.7	25.3	31.4	28.2	35.0	24.6	47.1
pH = 6.0	36.8	22.9	18.6	19.1	19.5	21.2	24.2	19.0	39.3
pH = 6.6	19.8	16.7	N.D.	N.D.	N.D.	19.4	19.6	17.3	24.2

N.D. = not detected

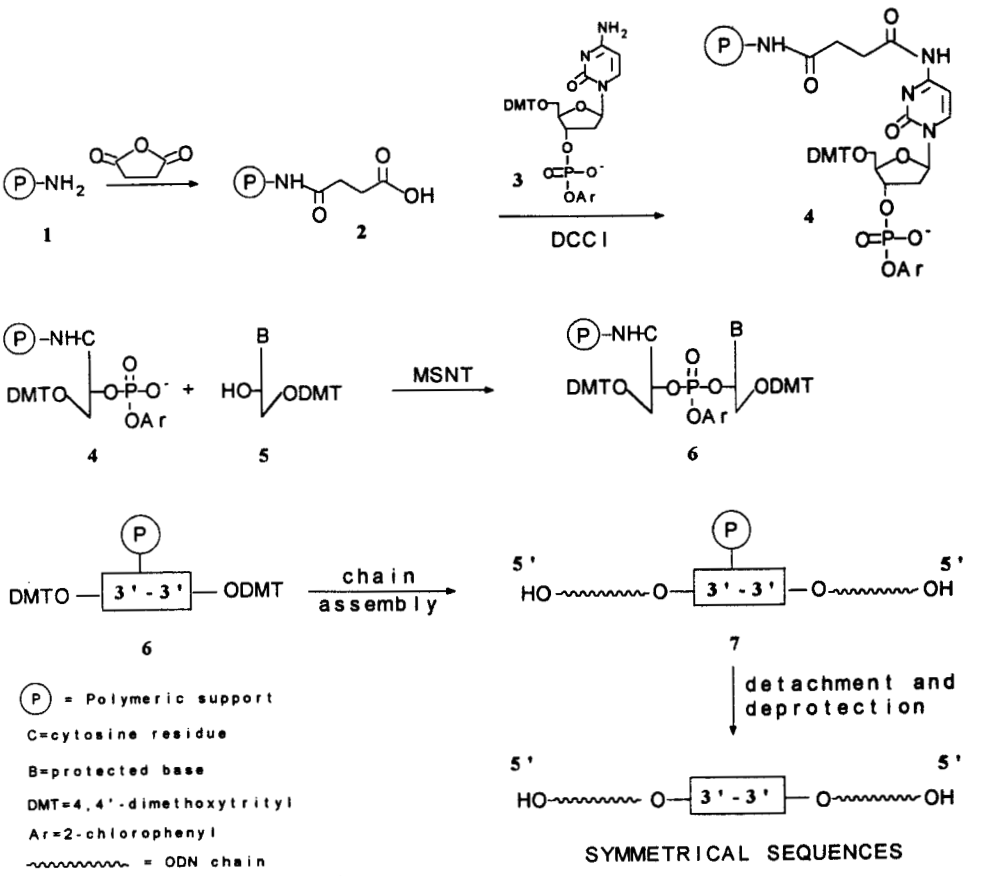
both the 3'- and the expensive 5'-phosphoramidites, uses a modified polymeric support linking the first nucleoside 3'-phosphodiester residue through the base (**4**, SCHEMES 1, 2). The 3'-phosphodiester moiety of functionalized support **4** could so be exploited to form the 3'-p-3' internucleosidic junction, while the 5'-OH group was used for the classical chain elongation. The desired inversion of polarity was introduced by coupling the 3'-phosphodiester function of **4** (or **8**) with the chosen 5'-DmTr-2'-deoxyribonucleoside (**5**), before the chain elongation (*symmetrical sequences*, SCHEME 1), or after the synthesis of the first 3'-5' tract (*asymmetrical sequences*,

SCHEME 2). For both the synthetic routes only 3'-phosphoramidites were required as building blocks in the chain assembly. Detachment and deprotection with conc. aq. ammonia yielded the desired oligonucleotides, which were purified and successively characterized by MALDI-TOF mass spectrometry.

Thermal denaturation experiments and gel retardation assays provided evidence that these oligomers are able to form intermolecular triple helical complexes by alternate strand recognition of duplexes having adjacent stretches of homopurines and homopyrimidines. By comparing the Tm values of selected 16-mers (***a-g***) mixed in 1:1 ratio with the complementary duplexes **I-III**, a marked dependence of stability of the resulting triplexes with sequence effects was found. Particularly, sequence ***a***, with alternated C's and T's, showed a good binding with 16-bp duplex **I**, almost comparable to that exhibited, in the same salt conditions, by a "natural" polypyrimidine ODN of the same length forming a "classical" triplex structure (***nat***), thus demonstrating that these modified ODNs can efficiently and cooperatively recognize two adjacent blocks of purines on both strands of the duplex. On the other hand, 16-mers containing couples of contiguous C's (***c***, ***d***, ***f*** and ***g***) resulted in a diminished affinity towards the complementary duplexes, which reflects the destabilization due to adjacent protonated cytosines.

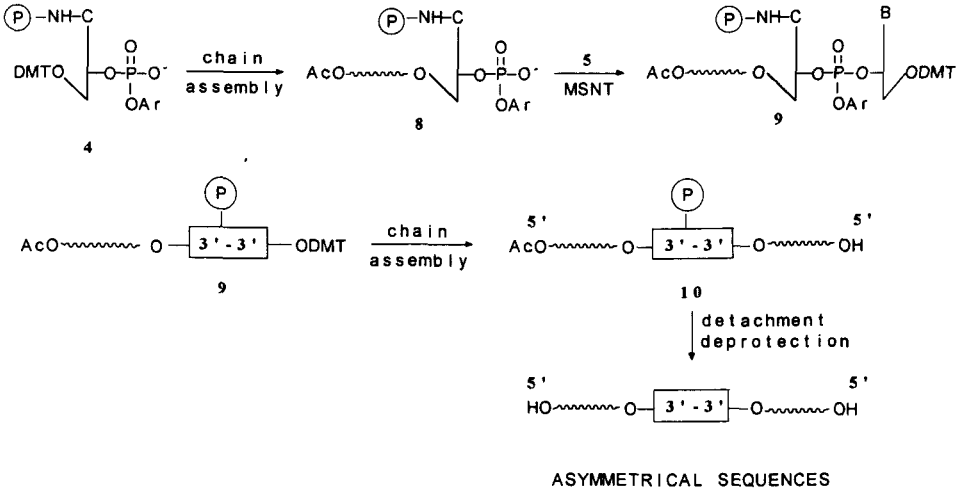
Specific "bridge" effects were also investigated, to determine whether the two nucleosides connected through the 3'-3' phosphodiester linkage were effectively involved in some kind of recognition process or acted merely as spacers linking the two 3'-termini of the third strand. Substitution of a C residue with a T at the inversion site, targeting a GC/CG box of the duplex, caused in all the studied cases a slight but detectable decrement of the Tm values. A not total disruption of the triplex structure at the 3'-3' junction was so hypothesized; point modifications of the bases flanking the 3'-3' bridge seemed to confirm that the 3'-3' linked nucleosides were to some extent involved in hydrogen bonding with the target W-C duplex, thus contributing to the total stabilization of the triple helical complex.

With the aim of giving a further insight into alternate strand triple helix formation and, particularly, of clarifying the structural details at the inversion site level, we have undertaken an NMR study of selected sequences. In order to get ^{1}H-NMR spectra as simplest as possible, our original idea was to investigate smaller molecules than those presented above, such as the complex formed by symmetrical 12-mer ***i*** with the duplex segment **IV** of the same lenght. However, the reduced stability of the resulting triplex would have forced us to work at impractically low temperatures. Therefore, we have turned our attention to the triplex deriving from symmetrical 16-bp duplex **V** and 16-mer ***l*** (FIGURE 1).



SCHEME 1

Preliminarly, we have been studying duplex **V**, thus assigning most of the exchangeable and non exchangeable signals through the well established methodologies based on NOESY (FIGURE 2) and DQF-COSY experiments[4]. The free duplex is symmetrical and, consequently, only one set of signals for the two strands was observed. We have then performed spectra of duplex **V** in the presence of variable amounts of *l*. The addition of increasing amounts of *l* (with saturation occurring at one equivalent) resulted in the falling off of the resonances attributable to the free duplex with the concomitant growing of a new set of signals, confidently assigned to the triple helix. The co-presence of the signals due to the free duplex and the triplex clearly indicated the complex to be kinetically stable on the NMR time scale. At the 1:1 *l* - **V** molar ratio, the signals of the free duplex completely disappeared. FIGURE 3 shows spectra acquired at several points of the titration experiment in H_2O.



SCHEME 2

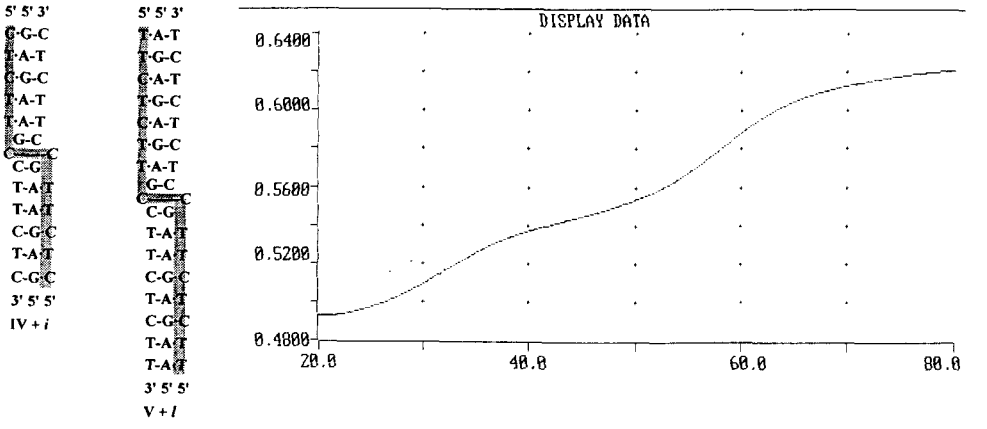


FIG.1: Melting profile of 16-mer **I** with the target duplex **V**. Buffer: 5 mM $MgCl_2$, 140 mM KCl, 5 mM NaH_2PO_4, pH=6.0; c≈ 1mM each strand; temperature increment 0.5°C/min.

5'-$A_1A_2G_3A_4G_5A_6A_7G_8C_9T_{10}T_{11}C_{12}T_{13}C_{14}T_{15}T_{16}$-3'

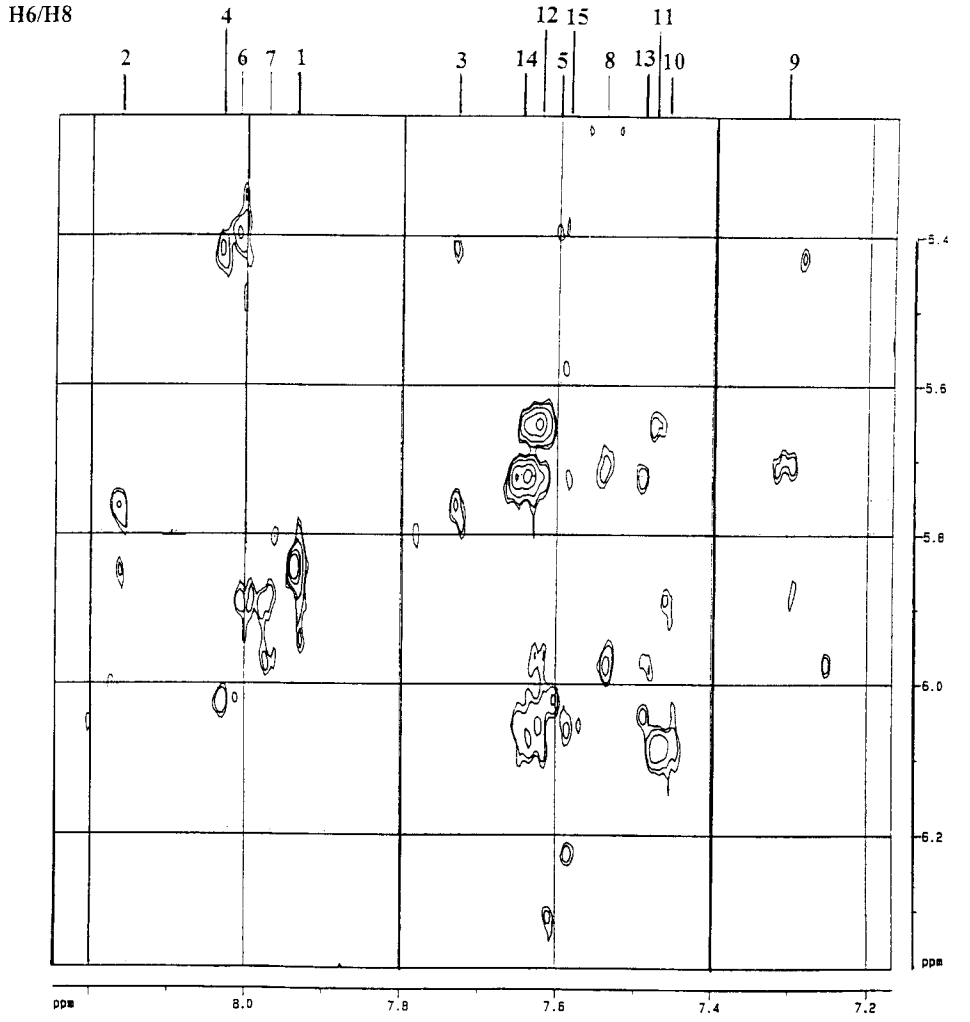


FIG.2: Base proton to sugar H1' proton region of 500 MHz NOESY spectrum in D_2O recorded at 298 K at 200 ms mixingtime of the duplex **V**.

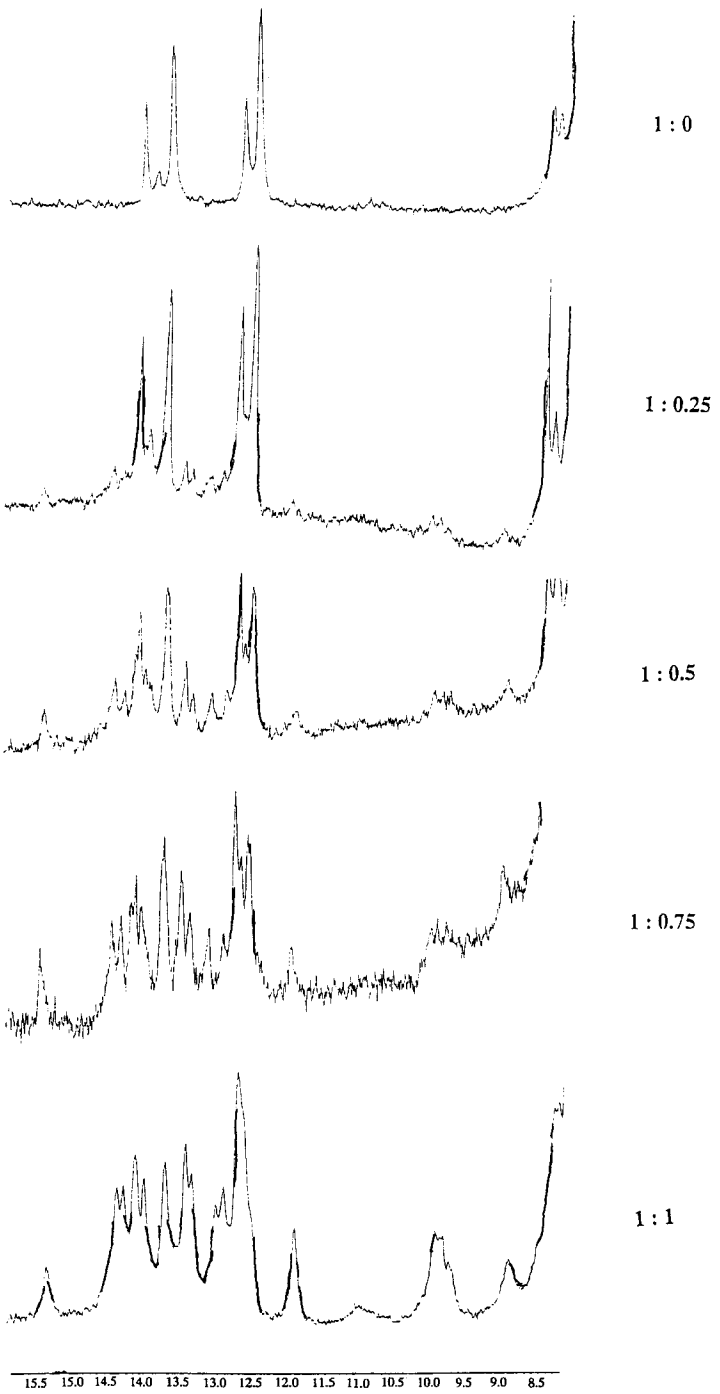


FIG. 3: Imino region of the 500 MHz ^{1}H-NMR spectra acquired at several points in the titration of duplex **V** with the oligo **1**, carried out in H2O:D2O 9:1 at 298K; the number to the right of the spectra refer to the duplex:oligo (3'-3') ratios

A detailed ^{1}H-NMR analysis of the triplex strucure is still in progress. However, two important features clearly emerged from these preliminary data. First, the triplex retains the initial two-fold symmetry of the duplex, as results from the number of lines present in the ^{1}H-NMR spectra in D_2O. Furthermore, the number of resonances due to the hydrogen-bonded imino protons in the ^{1}H-NMR spectrum in H_2O points to a reduction of fraying, when compared with the free duplex, and to a participation of the 3'-3' linked nucleosides present at the inversion site to the overall stabilization of the triple helical complex.

Acknowledgement.
We are grateful to MURST and CNR - "Progetto strategico: Nucleotidi antisenso come strumento di nuove strategie terapeutiche" for grants in support of this investigation.

REFERENCES

1) For a general review see: Thuong, N.T.; Helene, C. *Angew. Chem. Int. Ed. Engl.*, **1993**, *32*, 666;
2) Horne, D.A.; Dervan, P.B. *J. Am. Chem. Soc.*, **1990**, *112*, 2435; Ono, A.; Chen, C.N.; Kan, L. *Biochemistry*, **1991**, *30*, 9914; Froehler, B.C.; Terhorst, T.; Shaw, J.P.; McCurdy, S.N. *Biochemistry*, **1992**, *31*, 1603;
3) De Napoli, L.; Messere, A.; Montesarchio, D.; Pepe, A.; Piccialli, G.; Varra, M. *J. Org. Chem.*, in press;
4) Roberts, G.C.K., Ed., NMR of Macromolecules, The Practical Approach Series, IRL Press, Oxford, UK, 1993.