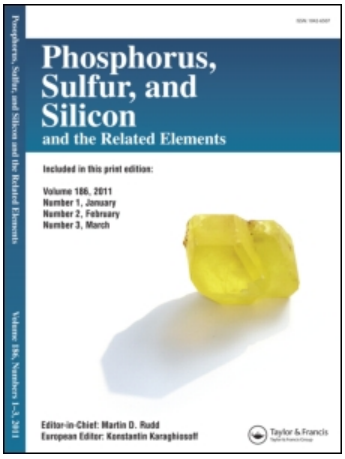


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

The Synthesis of Novel Modified Nucleic Acid Monomers and their Subsequent Solid Phase Oligomerisation to Give 3'-Methylene Phosphonate DNA (3MP)

Stephen P. Collingwood; Mark E. Douglas; Francois Natt; Uwe Pieles

To cite this Article Collingwood, Stephen P. , Douglas, Mark E. , Natt, Francois and Pieles, Uwe(1999) 'The Synthesis of Novel Modified Nucleic Acid Monomers and their Subsequent Solid Phase Oligomerisation to Give 3'-Methylene Phosphonate DNA (3MP)', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 645 — 648
To link to this Article: DOI: 10.1080/10426509908546327
URL: http://dx.doi.org/10.1080/10426509908546327

Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 645-648
Reprints available directly from the publisher
Photocopying permitted by license only

The Synthesis of Novel Modified Nucleic Acid Monomers and their Subsequent Solid Phase Oligomerisation to Give 3'-Methylene Phosphonate DNA (3MP)

STEPHEN P. COLLINGWOOD[a], MARK E. DOUGLAS[a], FRANCOIS NATT[b] and UWE PIELES[b]

[a]*Novartis Pharmaceuticals, Horsham Research Centre, Wimblehurst Road, Horsham, UK and* [b]*Novartis Pharmaceuticals, Basle, Switzerland*

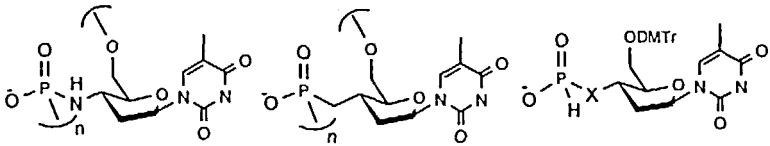
A protected hypophosphorus acid synthon allowed selective monoalkylation of hypophosphorus acid with 3'-iodo-methylene thymidine. The resulting H-phosphonous acid was an effective monomer for the solid phase synthesis of 3MP DNA which displayed strong binding to complementary RNA

Keywords: antisense; DNA; solid phase synthesis

The synthesis of nucleoside analogues for incorporation into oligomeric nucleic acids and consequent studies of their binding to complementary strands of DNA and RNA has been the focus of many laboratories over the last decade.[1] 3'-Phosphonamidate DNA (3NP) **1**[2] has been of particular recent interest due to its strong affinity for

complementary RNA. The degree of stabilisation found for hybrid 3NP-RNA duplexes has in large part been assigned to a bias towards 3'endo ring pucker of the furanose ring. In contrast to 3NP **1** 3'-methylene phosphonate DNA **2** (3MP)[3], where similar arguments in favour of binding to RNA can be made, has remained uninvestigated with regard to its effect as an antisense oligonucleotide analogue. We felt that these derivatives should have increased hydrolytic stability in comparison to 3NP and similar biophysical properties.

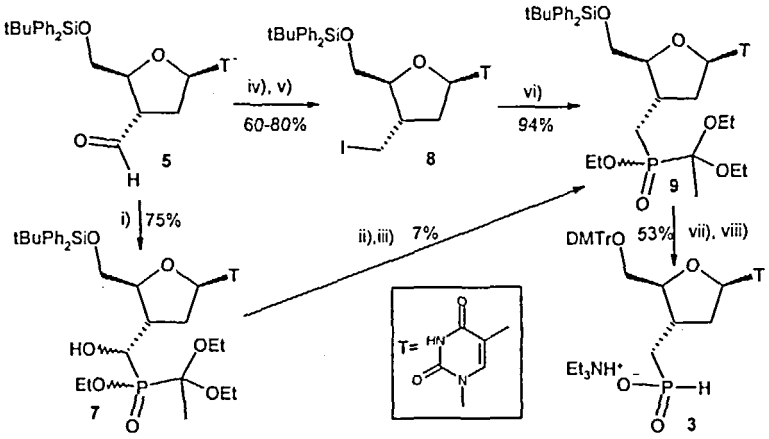
The simple chemical relationship between 3MP and normal DNA suggested the use of the novel H-phosphonous acid **3**, as monomer



1 **2**

for solid phase DNA synthesis. This acid is a direct analogue of the well established H-Phosphonate DNA synthesis monomer **4**.[4] Initially we followed an approach, similar to our studies in phosphinate DNA[5], utilising the readily available thymidine aldehyde **5**.[6] Addition of the hypophosphorus acid synthon **6** to aldehyde **5** giving phosphinate **7** in good yield. However deoxygenation proved troublesome due to the sterically congested environment of the 3' substituent. Fortunately the alternative Arbusov reaction of the potassium anion of hypophosphorus acid synthon **6** with the iodide **8** progressed in excellent yield. All protection was removed from the resulting methylene phosphinate **9** by aqueous acidic hydrolysis. Standard tritylation then gave the required DMTr protected derivative **11** ready for oligomerisation studies. A comparison of monomer **3** versus the normal H-phosphonate monomer **4** under standard H-Phosphonate

coupling conditions (pivaloyl chloride in pyridine/ acetonitrile) showed a substantially reduced reactivity for **3** (*ca* 100x less reactive than **4**).



i) 1.15 equiv. $Me(EtO)_2CP{=}O(H)(OEt)$ **6**, 1 equiv. DBU, THF, rt, 1h; ii) 2.3 equiv. TolOCSCl, DMAP, Et_3N, CH_2Cl_2, rt-65°C, 48h (ca 10% yield); iii) 1.2 equiv. Bu_3SnH, Toluene, 100°C, 18h; iv) $NaBH_4$, EtOH, rt, 18h;v) 1.15 equiv $MeP(OPh)_3I$, DMF, 2 equiv. lutidine, rt, 1h, or TsCl or MsCl, pyr, rt, followed by 4 equiv. NaI, acetone, reflux, 6h; vi) 5 equiv. $Me(EtO)_2CP{=}O(H)(OEt)$ **6**, 4 equiv. KHMDS, THF, -78°C to rt; vii) 2N HCl in $EtOH/H_2O$ (8:1), 50°C, 6h; viii) 1.1 equiv. DMTrCl, Et_3N, Pyr, rt, 5h.

This reduced reactivity is presumably related to the lower electronegativity of the 3' carbon versus oxygen. Oxidation and sulphurisation rates of the intermediate H-Phosphonous ester backbone were also substantially lower than for the normal H-phosphonate diester backbone. A resulting modified solid phase DNA synthesis protocol was developed involving double coupling with modified monomer (60 fold excess 2x 30 min coupling, >95% yield) and an extra oxidation step (0.2M Iodine Pyr:Et_3N:water 6:1:1). We were also able to prepare the novel 3'-methylene phosphonothioate modification by substituting the final oxidation step by sulphurisation (5% S_8 CS_2:Pyr:Et_3N 10:10:1).The oligonucleotides (table) were prepared in

small plastic syringes by hand and characterised by MALDI-TOF mass spectroscopy and their binding to complementary RNA investigated. The inclusion of a single phosphonate modification gave a substantial enhancement to binding affinity. Addition of a neighbouring 2'-methoxy DNA unit provided additional stabilisation (+1.2°C). The effect of the novel phosphonothioate modification was less readily assessed due to the lack of the corresponding comparison sequences, however the modification appears not to be destabilising relative to phosphorothioate DNA.

In conclusion 3MP DNA appears to be a promising antisense modification, further studies of this new antisense paradigm will be reported in due course.

Table

Oligonucleotide	δT_m (per modification) °C vs RNA relative to unmodified DNA
TTtTT	-
TTTtTCTCTCTCTCT	+2.9
TTT*t*CTCTCTCTCT	+2.05 (average, both modifications)
TsTsTstsTCsTsCsTsCsTsCsTsCsT	-0.7 (average, all modifications)
TsTsTsts*s*CsTsCsTsCsTsCsTsCsT	-0.4 (average, all modifications)

t= modified monomer, s refers to P=S backbone, *t*= 2' methoxy DNA

References

[1] S M Freier, K-H Altmann, *Nucleic Acids Res.*, 4429, **25** (1997).
[2] V Tereshko, S Gryaznov, M Egli, *J. Am. Chem Soc*, 269, **120**, (1998) and references therein.
[3] U Heinemann, L N Rudolph, C Alings, M Morr, W Heikens, R Frank, H Blocker, *Nucleic Acids Res.* 427, **19**, (1991) and references therein.
[4] B Froehler in *Protocols for Oligonucleotides and Analogues* Ed S Agrawal, Humana, (1993).
[5] S P Collingwood, A D Baxter, *Synlett*,703 (1995).
[6] Y S Sanghvi, R Bharadwaj, F Debart, A DeMesmaeker, *Synthesis*,1163, (1994).