Tetrahedron Letters,Vol.28,No.46,pp 5713-5716,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

(Butylthio)carbonyl Group: A New Protecting Group for the Guanine Residue in Oligoribonucleotide Synthesis

Masayo Fujii, Shunichi Yamakage, Hiroshi Takaku*, and Tsujiaki Hata+

Laboratory of Bioorganic Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275, Japan and +Department of Life Chemistry, Tokyo Institute of Technology, Midoriku, Nagatsuta, Yokohama 227, Japan

Summary: The protection of the O^6-amide and N^2-amino groups of guanosine with the (butylthio)carbonyl group is described. This group could be rapidly introduced in good yields and removed very easily under the conventional deprotective condition for the exo-amino acyl groups of other nucleoside bases.

As one of problems in oligonucleotide synthesis, the remaining reactive site of guanine residue is reacted with the condensing and phosphorylating agents commonly employed in oligonucleotide synthesis to give the O^6-sulfonylated and phosphorylated guanosine derivatives as the side reaction products.[1] Several protecting groups have recently been proposed to prevent the side reaction.[2] Recently, we found that the (butylthio)carbonyl (BTC) group was most effective as a protecting group for the O^3-imide group of the uracil residue in the ribo-series.[3] In this paper, we wish to report the protection of the O^6-amido and N^2-amino groups of guanosine utilizing the (butylthio)carbonyl group and its application to the synthesis of GACCGUCA, box 9R' sequence of r-RNA precurson of Tetrahymena[4].

We first investigated introduction of the (butylthio)carbonyl group on the guanine residue: Guanosine (1) (2.83 g, 10 mmol) was treated with trimethysilyl chloride (TMSCl) (12.8 ml, 100 mmol) in dry pyridine (50 ml) for 1 h; subsequently butyl chlorothioformate (BuSCOCl) (8.4 ml, 60 mmol) and diisopropylethylamine (iPr_2NEt) (10.4 ml, 60 mmol) were added and the reaction mixture was kept for 24 h at room temperature. After the usual workup, silica gel column chromatography using a mixture of CH_2Cl_2 and MeOH (95:5, v/v) eluants afforded compound 2[5] (3.61 g, 70%). The silylated nucleoside 3 was prepared in 80% yield by the treatment of 2 with 1,3-dichloro-1,1,3,3-tetraisopropyldisloxane ($TIPDSiCl_2$) in pyridine. Compound 3 was treated with 2,3-hydropyran in the presence of $TsOH.H_2O$ in dioxane for 3 h to give 4, which was then desilylated to 5[6] in 75% yield by 1M tri-1-butylammonium hydrogen fluoride (TBAHF). Treatment of 5 with DMTrCl in dry pyridine gave the expected 5'-tritylated product 6 in good yield. Other nucleoside derivatives (7) were prepared by according to our methodology.[3,7]

Next, the fully protected mononucleotide units (9) were obtained in 84-87% yields by treatment of the nucleoside derivatives (6,7) with the phosphorylating agent 8 prepared simply from 2,6-dichlorophenyl phosphorodichloridate and 5-chloro-8-hydroxyquinoline in the one flask reaction (Scheme 1).[8]

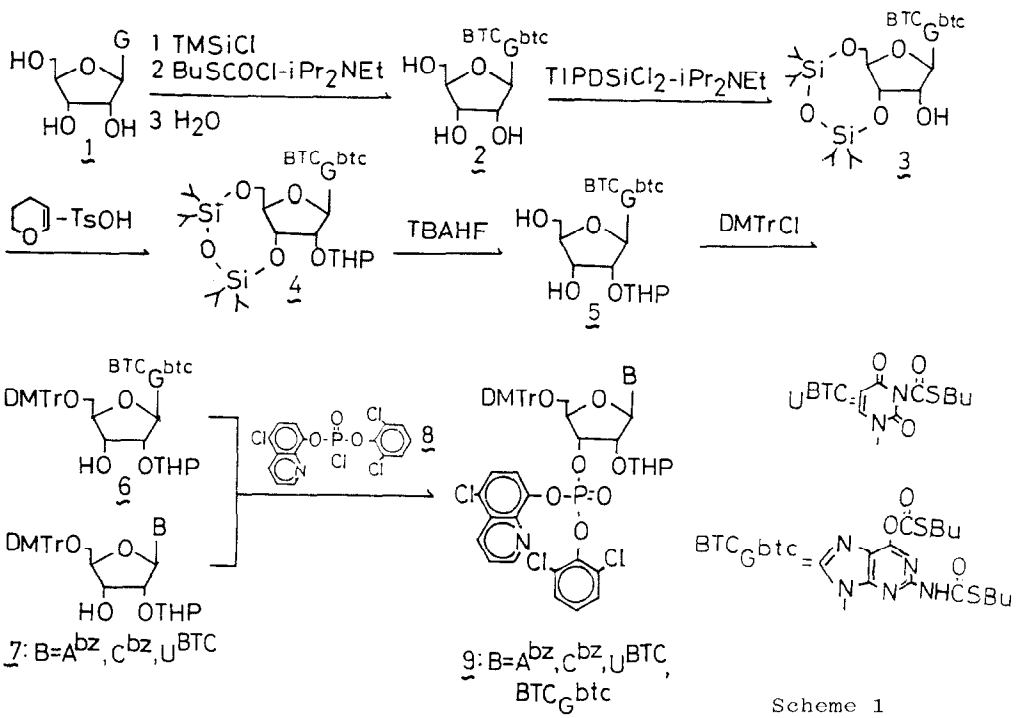
In this phosphorylation, no loss of the guanine and uracil protecting groups was observed.

Table 1. Stabilities of the Guanosine derivative Under Various conditions[a)]

Compound	A	B	C	D	E	F
2	stable	stable	stable	stable	stable	98%

a) (A) 2% TsOH in CH_2Cl_2-MeOH (7:3, v/v), room temperature, 30 min; (B) 80% AcOH, room temperature, 2 h; (C) Et_3N-CH_3CN (1:1, v/v), room temperature, 1 day; (D) pyridine-t-$BuNH_2$-H_2O (8:1:1, v/v), room temperature, 1 day; (E) 1M TBAHF in THF, room temperature, 1 day; (F) concentrated NH_4OH-MeOH (9:1, v/v), room temperature, 3 h.

It was then interesting to explore the relative stabilities of O^6- and N^2-(butylthio)carbonyl group in guanosine 2 under a variety of deprotective conditions to evaluate their possible use in the oligoribonucleotide synthesis in conjunction with other protecting groups on sugar and phosphotriester moieties. These results are summarized in Table I. Thus, it becomes clear from deprotective conditions (A, C, D, and F) in Table I that the (butylthio)-carbonyl group can be used in conjunction with other sugar and phosphate protecting groups in our approach to the oligoribonucleotide synthesis. In particular, compound 2 was treated with a mixture of conc. ammonia and methanol (9:1, v/v) at room temperature for 3 h to afford guanosine without the formation of 6-thiobutylguanosine (12)[9)] and 2,6-diamino-purine derivative (13)[10)].



Scheme 1

To demonstrate the utility of the (butylthio)carbonyl group, octaribonucleotide, GACCGUCA, i.e., box 9R' sequence of r-RNA precurson of Tetrahymena was synthesized. According to our approach to the oligonucleotide synthesis,[8] the mononucleotide units (9) were treated with pyridine-t-$BuNH_2$-H_2O (8:1:1, v/v) at room temperature for 3 h to give the corresponding phosphodiesters (10) as triethylammonium salt which were used in the next coupling reaction without further purification. On the other hand, treatment of 9 with 2% TsOH in CH_2Cl_2-MeOH (7:3, v/v) at 0 °C for 10 min followed by washing with 5% $NaHCO_3$ solution gave the 5'-hydroxyl components (11).[11] Compounds (10, 11) of thus obtained were used for the synthesis of octaribonucleotide by fragment condensation as shown in Fig. 1. The conditions and results of the fragment condensations are summarized in Table II.

The coupling reactions proceeded smoothly and high yield of the synthesis of octamer containing guanosine and uridine units.

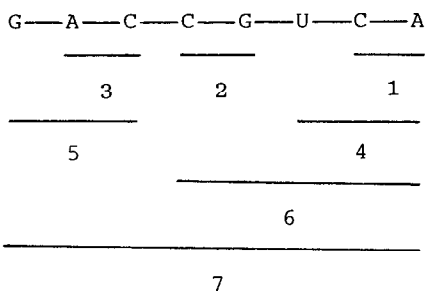
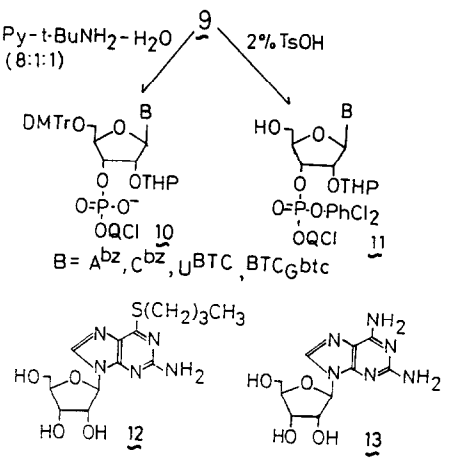


Fig. 1. Strategy for the synthesis of the octaribonucleotide.



Scheme 2

Table II. The conditions and results of fragment condensations for the synthesis of the fully protected octaoligoribonuceotide

3'-Diester compd (equiv.)	pyridine-t-$BuNH_2$-H_2O (8:1:1) Time/h	5'-OH compd	QSCl—MeIm equiv.		Time (h)	Frag. No.	yield (%)	Removal of DMTr group with 2% TsOH at 0°C Time/min	yield/%
Cp (1.5)	3	A	3.0	6.0	1	1	91		
Cp (1.5)	3	Gp	3.0	6.0	1	2	82	10	87
Ap (1.5)	3	Cp	3.0	6.0	1	3	86	10	90
Up (1.5)	3	CA	3.0	6.0	1.5	4	95	10	96
Gp (1.5)	3	ACp	3.0	6.0	1.5	5	81	10	86
CGp (1.7)	3	UCA	3.4	6.8	2.0	6	95	10	88
GACp (2.0)	4	CGUCA	4.0	8.0	2.0	7	98	12	88

Deprotection of the octamer GACCGUCA (15 mg, 2.5 umol) was performed as follows: 1) 0.3 M N^1,N^1,N^3,N^3-tetramethylguanidium syn-2-nitrobenzaldoximate (TMG-NBO) in dry CH_3CN at 30 °C for 1 day to remove the 5-chloro-8-quinolyl groups;[12] 2) conc. ammonia-pyridine (9:1, v/v) at 60 °C for 12 h to remove the benzoy and (butylthio)carbonyl groups; 3) 0.01 M HCl in dioxane-H_2O (1:1, v/v) at room temperature for 2 days to remove the THP and DMTr groups. Finally, purification by dialysis tubing (Medical International LTD) and reversed phase HPLC (Senshu C_{18}) gave the pure octamer GACCGUCA (2.5 OD). The octamer was digested by snake venom phosphodiesterase and alkaline phosphatase to give mononucleosides, C, U, G, and A (2.95:1.00:2.18:2.12). Furthermore, a sample thus obtained was characterized by 20% polyacrylamide gel electrophoresis in the presence of 7 M urea (a single spot was obtained and it was a octamer).

Acknowledgment We thank Dr Mitsuo Sekine for his encouragement throughout the ivestigation.

References and Notes

1) C. B. Reese and Safhill, J. Chem. Soc. Chem. Commun., 1968, 767; C. B. Reese and A. Ubasawa, Tetrahedron Lett., 21, 2265 (1980); H. P. Daskalov, M. Sekine, and T. Hata, ibid., 21, 3899 (1980); idem., Bull. Chem. Soc. Jpn., 54, 3076 (1981); S. S. Jones, C. B. Reese, S. Sibanda, and A. Ubasawa, Tetrahedron Lett., 22, 4755 (1982); J. A. der Hartog, G. Wille, R. A. Scheublin, and J. H. van Boom, Biochemistry, 21, 1009 (1982); H. Takaku, K. Kamaike, and K. Kasuga, Chem. Lett., 1982, 197; E. Ohtsuka, A. Yamane, and M. Ikehara, Nucleic Acids Res., 11, 1325 (1983).

2) S. S. Jones, C. B. Reese, S. Shibanda, and A. Ubasawa, Tetrahedron Lett., 22, 4755 (1981); B. L. Gaffney and R. A. Jones, ibid., 23, 2257 (1982); B. E. Watkins, J. S. Kieyl, and H. Rapoport, J. Am. Chem. Soc., 104, 5702 (1982); M. Sekine, J. Matsuzaki, T. Hata, Tetrahedron Lett., 23, 5287 (1882); T. Kamimura, M. Tsuchiya, K. Koura, M. Sekine, and T. Hata, ibid., 24, 2775 (1983); T. Trichtinger, R. Charubala, and W. Pfleiderer, ibid., 24, 711 (1983); H. Takaku, S. Ueda, and Y. Tomita, Chem. Pharm. Bull., 32, 2885 (1983); C. B. Reese and P. A. Skone, J. Chem. Soc. Perkin I, 1984, 1263; X.-X. Zhou, I. Ugi, J. Chattopadhyaya, Acta Chem. Scand., B 40, 761 (1985).

3) M. Fujii, Y. Horinouchi, and H. Takaku, Chem. Pharm. Bull., in press.

4) H. R. Cech, N. K. Tanner, I. Tinoco, Jr., B. R. Weir, M. Zuker, and P. S. Perlman, Proc. Natl. Acad. Sci. USA, 80, 3903 (1983).

5) UV max (MeOH) 296, 237 nm, min (MeOH) 256 nm. ^{1}H-NMR ($CDCl_3$): 8.40 (s, 1H, H-8), 5.98 (d, 1H, $J_{1',2'}$=6 Hz, H-1'), 4.78 (d, 1H, H-2'), 4.60 (m, 3H, HO-2', HO-3', HO-5'), 4.37 (m, 1H, H-3'), 4.20 (br s, 1H, H-4'), 3.80 (br s, H-5'), 2.90 (br s, 4H, SCH_2), 1.52 (m, 8H, CH_2), 0.95 (t, 6H, CH_3). Anal. Calcd for $C_{20}H_{29}O_7N_5S_2$: C, 48.88; H, 6.66; N, 11.37; Found: C, 48.87; H, 6.66; N, 11.33.

6) UV max (MeOH) 296, 285 (sh), 236 nm, min (MeOH) 267 nm. ^{1}H-NMR ($CDCl_3$): 8.79 (s, 1H, H-8), 6.32 (d, 1H, $J_{1',2'}$=6 Hz, H-1'), 5.23 (d, 1H, H-2'), 4.90-4.70 (m, 3H, H-3', H-4', acetal proton of THP), 4.48 (m, 2H, HO-3', HO-5'), 4.07 (m, 2H, H-5'), 3.60 (m, 8H, SCH_2, O-methylene of THP), 2.10-1.60 (m, 12H, CH_2, C-methylene of THP), 1.20 (t, 6H, CH_3). Anal Calcd for $C_{25}H_{37}O_8N_5S_2.1/2C_6H_{14}$: C, 52.31; H, 6.89; N, 10.87. Found: C, 52.47; H, 6.73; N, 10.64.

7) H. Takaku, M. Yoshida, and T. Nomoto, J. Org. Chem., 4, 1399 (1983).

8) H. Tsuchiya, S. Ueda, H. Kobayashi, and H. Takaku, Chem. Lett., 1985, 347.

9) B. E. Watkins and H. Ropoport, J. Org. Chem., 47, 4471 (1982).

10) P. K. Bridson, W. Markiewicz, and C. B. Reese, J. Chem. Soc. Chem. Commun., 1977, 447.

11) H. Takaku, R. Yamaguchi, T. Nomoto, and T. Hata, Tetrahedron Lett., 1979, 3857.

12) K. Kamaike, S. Ueda, H. Tsuchiya, and H. Takaku, Chem. Pharm. Bull., 31, 2928 (1983).

(Received in Japan 11 July 1987)