New synthetic routes to synthons suitable for 2'-O-allyloligoribonucleotide assembly

Brian S.Sproat*, Adolfo M.Iribarren[1], Ramon Guimil Garcia and Barbro Beijer
European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG and [1]Instituto di Ricerche di Biologia Molecolare, Via Pontina, Km 30.600, 00040 Pomezia, Italy

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ABSTRACT

New synthetic routes have been devised for the high yield preparation of protected 2'-O-allylribonucleoside-3'-O-phosphoramidites, exemplified by the ribonucleosides guanosine and 2,6-diaminopurine riboside (2-aminoadenosine). Key features are the use of versatile intermediates and an easy allylation step. The development of a novel synthon based on 2'-O-allyl-2,6-diaminopurine riboside enables short 2'-O-allyloligoribonucleotide probes to be synthesized with adenine replaced by 2-aminoadenine. Thus very stable hybrids with complementary RNA target sequences can be formed due to the formation of the three hydrogen bond 2-amino A·U base pairs.

INTRODUCTION

We have recently described the application of oligo(2'-O-allylribonucleotides) as superior antisense compounds for investigating RNA processing (1). Initially the monomers were prepared in identical fashion to the 2'-O-methyl analogues (2) except that allyl bromide was used instead of methyl iodide for the alkylation step. Thus highly selective 2'-O-allylation of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) ribonucleosides carrying appropriate protection of the heterocyclic base could be achieved using allyl bromide and 2-tert.-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin (BDDDP) in acetonitrile. Isolated yields were in the range 60–65% with a reaction time of 5 h at room temperature. Silver oxide catalysed alkylation (3) was only successful for the pyrimidine derivatives, a reaction time of 48 h at room temperature gave yields in the range 60–70%. Although problems were encountered in the preparation of the 2'-O-allylguanosine monomer, we describe here how these have now been solved.

Here we describe high yield syntheses of protected 2'-O-allylribonucleoside-3'-O-phosphoramidites based on a rapid and inexpensive allylation procedure. Moreover the use of synthetically versatile key intermediates allowed us to prepare a 2'-O-allyl-2,6-diaminopurine riboside building block.

EXPERIMENTAL

General Materials and procedures

Tris(dibenzylideneacetone)dipalladium(0) and 1,4-bis(diphenylphosphino)butane were obtained from Aldrich (Steinheim, Germany). 2-Cyanoethoxy N,N-diisopropylaminochlorophosphine was purchased from BioSyntech (Hamburg, Germany) and calf intestinal adenosine deaminase was obtained from Sigma (Deisenhofen, Germany).

Phenoxyacetic anhydride was prepared as described in the literature (24). 3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-2-chloro-6-(2,6-dichlorophenoxy)purine riboside was prepared as previously described (2). All other reagents used were of the highest available purity. Anhydrous solvents were purchased from Romil Chemicals Ltd (Loughborough, England).

In general the nucleoside derivatives were purified either by preparative liquid chromatography using silica cartridges and a Waters system 500A liquid chromatograph (Waters Millipore, Milford, USA) or by column chromatography on Kieselgel 60 (Fluka, Neu-Ulm, Germany). Ascending mode t.l.c. was performed on aluminium foil supported silica gel containing a 254 nm fluor.

^{13}C and ^{31}P n.m.r. spectra were recorded on a Bruker AM250 spectrometer using tetramethylsilane and external trimethyl phosphate as the respective references. ^{31}P n.m.r. spectra were recorded using broad band proton noise decoupling. ^{13}C n.m.r. data are reported below with broad band proton noise decoupling, however assignments were made using the off resonance data. ^{13}C n.m.r. data for the 5'-O-dimethoxytrityl compounds and the 3'-O-phosphoramidites are not included, but are available upon request. Full synthetic details and n.m.r. spectroscopic data for the 2'-O-allyluridine, cytidine, adenosine and inosine monomers are also available upon request.

Synthesis of the Allylation Reagent and Monomers

Allyl ethyl carbonate. Dry pyridine (79.1 g, 1 mol) and absolute ethanol (87.5 ml, 1.5 mol) were mixed and cooled to 0°C under dry argon. Allyl chloroformate (120.5 g, 1 mol) was added dropwise with stirring under anhydrous conditions. Upon completion of addition the reaction mixture was stirred for 2 h at room temperature. Dry ether (1 l) was added with stirring and the pyridine hydrochloride was removed by filtration. The filtrate

* To whom correspondence should be addressed

was washed with 80% saturated brine, dried (Na_2SO_4), filtered and solvent was removed *in vacuo* at room temperature. Allyl ethyl carbonate was obtained as a pale yellow liquid (118.4 g, 91%) and was stored under argon at 4°C. ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 154.68 (C=O), 131.51 (allyl CH), 118.20 (=CH_2), 67.86 (allyl CH_2-O), 63.63 (ethyl CH_2) and 13.92 ppm (CH_3).

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-2'-O-allyl-2-chloro-6-(2,6-dichlorophenoxy)purine riboside (II). Tris(dibenzylideneacetone)dipalladium(0) (192 mg, 0.21 mmol) and 1,4-bis(diphenylphosphino)butane (358 mg, 0.84 mmol) were suspended in dry tetrahydrofuran (42 ml) under argon. A solution of compound I (14.49 g, 21 mmol) and allyl ethyl carbonate (5.46 ml, 42 mmol) in dry tetrahydrofuran (63 ml) was added, and the mixture was refluxed under argon for 30 min. Silica gel t.l.c. in petrol/ethyl acetate (4:1 v/v) showed complete reaction. The reaction mixture was cooled, filtered and solvent was removed *in vacuo*. The crude product was purified by preparative liquid chromatography on a single silica cartridge using petrol/ethyl acetate (9:2 v/v) as eluant. The title compound was obtained as a pale yellow foam (13.7 g, 89.4%) of R_f 0.31 on t.l.c. in petrol/ethyl acetate (4:1 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 158.20 (C-6), 153.19 (C-2), 152.64 (C-4), 144.82 (phenyl C-1), 142.26 (C-8), 133.93 (allyl CH), 128.95 (phenyl C-2 and C-6), 128.65 (phenyl C-3 and C-5), 127.19 (phenyl C-4), 120.63 (C-5), 117.66 (allyl =CH_2), 88.77 (C-1'), 81.50 (C-2'), 80.48 (C-4'), 71.71 (allyl CH_2O), 69.17 (C-3'), 59.68 (C-5'), 17.32–16.76 (isopropyl CH_3s), 13.28, 12.81 and 12.47 ppm (isopropyl CHs).

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-2'-O-allyl-2,6-diazidopurine riboside (III). Compound II (13.45 g, 18.4 mmol) and sodium azide (3 g, 46 mmol) were stirred in dry N,N-dimethylformamide (400 ml) under argon for 6 h at 55°C. The solution was allowed to cool overnight and solvent was removed *in vacuo*. The residual dark oil was dissolved in dichloromethane (500 ml) and the solution washed with water (500 ml), dried (Na_2SO_4), filtered and solvent removed *in vacuo*. The residue was purified by column chromatography on silica gel (220 g) using petrol/ethyl acetate (5:1 v/v) as eluant. The title compound was obtained as a pale yellow oil (8.95 g, 79%) of R_f 0.16 on t.l.c. in petrol/ethyl acetate (4:1 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 155.40 (C-6), 153.04 (C-2), 151.94 (C-4), 141.07 (C-8), 133.73 (allyl CH), 121.47 (C-5), 116.78 (allyl =CH_2), 88.09 (C-1'), 81.18 (C-2'), 80.52 (C-4'), 71.14 (allyl CH_2O), 68.53 (C-3'), 59.33 (C-5'), 16.96–16.36 (isopropyl CH_3s), 12.95, 12.51 and 12.18 ppm (isopropyl CHs).

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-2'-O-allyl-2,6-diaminopurine riboside (IV). Anhydrous stannous chloride (8.25 g, 43.5 mmol) was dissolved with stirring in dry acetonitrile (290 ml) and then thiophenol (17.9 ml, 174 mmol) and triethylamine (18.15 ml, 130.5 mmol) were added. Compound III (8.95 g, 14.5 mmol) dissolved in dry acetonitrile (50 ml) was added to the above yellow solution. T.l.c. in 5% ethanol/chloroform showed complete reaction after 15 min. Solvent was removed *in vacuo* and the yellow residue was dissolved in dichloromethane (300 ml). The solution was washed with 1 M aqueous sodium hydroxide (300 ml) and the aqueous phase was backwashed with dichloromethane (2×200 ml). The combined organic layers, now colourless, were dried (Na_2SO_4), filtered and solvent was removed *in vacuo*. T.l.c. in 5% ethanol/dichloromethane showed a single spot of R_f 0.16. The crude product was purified by column chromatography on silica gel (200 g), eluting with a gradient of ethanol from 0 to 5% in dichloromethane. The title compound was obtained as a solid white foam (7.66 g, 93%). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 159.87 (C-6), 155.80 (C-2), 150.30 (C-4), 134.80 (C-8), 133.96 (allyl CH), 116.90 (allyl =CH_2), 113.83 (C-5), 87.62 (C-1'), 80.83 (C-2'), 80.66 (C-4'), 71.27 (allyl CH_2O), 68.78 (C-3'), 59.57 (C-5'), 17.04–16.46 (isopropyl CH_3s), 13.04, 12.55 and 12.22 ppm (isopropyl CHs).

2'-O-Allyl-2,6-diaminopurine riboside (V). Compound IV (4.55 g, 8.05 mmol) was dissolved in dry tetrahydrofuran (20 ml) and 1.1 M tetrabutylammonium fluoride in tetrahydrofuran (18 ml) was added with stirring and exclusion of moisture. Silica gel t.l.c. in ethanol/chloroform (1:4 v/v) showed complete reaction after 5 min. The reaction mixture was quenched with pyridine/methanol/water (50 ml, 3:1:1 v/v/v), and the solution was poured into stirred pyridinium form Dowex 50 W×4-200 resin (30 g) suspended in pyridine/methanol/water (50 ml, 3:1:1 v/v/v). The mixture was stirred for 20 min, the resin filtered off and washed with the above solvent mixture (3×50 ml). Combined filtrate and washings were evaporated to dryness *in vacuo*, and the residual glass was dried by evaporation of toluene. The crude product was purified by column chromatography on silica gel (100 g) eluting with a gradient of ethanol from 5 to 15% in dichloromethane. Pure title compound was obtained as a solid white foam (2.5 g, 96%) of R_f 0.45 on t.l.c. in ethanol/dichloromethane (1:4 v/v). ^{13}C n.m.r. spectrum (DMSO-d_6) δ: 160.20 (C-6), 156.43 (C-2), 151.39 (C-4), 136.57 (C-8), 134.73 (allyl CH), 116.92 (allyl =CH_2), 113.67 (C-5), 86.50 (C-1'), 85.81 (C-4'), 80.47 (C-2'), 70.43 (allyl CH_2O), 69.46 (C-3') and 61.86 ppm (C-5').

N^2-Dimethylaminomethylidene-2'-O-allylguanosine (VI). Compound V (2.5 g, 7.75 mmol) was dissolved in a mixture of dimethylsulphoxide (66 ml) and 0.1 M aqueous sodium phosphate (160 ml, pH 7.5). Crude adenosine deaminase (150 mg) was added and the solution was shaken gently at 37°C for 72 h. Silica gel t.l.c. in ethanol/dichloromethane (1:4 v/v) showed complete reaction with a new spot at R_f 0.25 due to 2'-O-allylguanosine. Solvent was removed *in vacuo*. The residue was suspended in methanol (100 ml), N,N-dimethylformamide dimethyl acetal (5 ml, 38 mmol) was added, and the mixture was stirred overnight at room temperature. T.l.c. in ethanol/dichloromethane (1:4 v/v) showed complete reaction with a new spot of R_f 0.30. The reaction mixture was evaporated to dryness *in vacuo* and further dried by evaporation of toluene. Purification by column chromatography on silica gel (100 g) eluting with a gradient of ethanol from 5 to 20% in dichloromethane afforded the title compound as a solid white foam (2.90 g, 99%). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 157.96 (C-6 and amidine CH), 156.79 (C-2), 149.11 (C-4), 139.94 (C-8), 133.38 (allyl CH), 120.45 (C-5), 117.59 (allyl =CH_2), 87.46 (C-1'), 86.08 (C-4'), 80.53 (C-2'), 71.28 (allyl CH_2O), 67.77 (C-3'), 61.67 (C-5'), 41.03 and 34.72 ppm (N-CH_3s).

5'-O-Dimethoxytrityl-N^2-dimethylaminomethylidene-2'-O-allylguanosine (VII). Compound VI (2.90 g, 7.6 mmol) was dried by evaporation of pyridine (2×20 ml) *in vacuo*. Anhydrous pyridine (30 ml), triethylamine (2.1 ml, 15 mmol) and 4,4'-dimethoxytrityl chloride (3.39 g, 10 mmol) were added with stirring and exclusion of moisture. Silica gel t.l.c. in triethylamine/ethanol/chloroform (1:10:89 v/v/v) showed

complete reaction after 1 h. The reaction was quenched by addition of methanol (1 ml) and solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (100 ml) and the solution washed with 1 M aqueous sodium bicarbonate (2×100 ml). The organic phase was separated, dried (Na_2SO_4), filtered and solvent was removed *in vacuo*. Residual pyridine was removed by evaporation of toluene. The crude product was purified by column chromatography on silica gel (100 g). Elution was performed initially with dichloromethane/ethyl acetate (1:1 v/v) containing 0.5% triethylamine, and then with a gradient of ethanol from 0 to 8% in ethyl acetate/triethylamine (199:1 v/v). The title compound was obtained as a solid white foam (5.14 g, 99%) of R_f 0.15 on t.l.c. in triethylamine/ethanol/dichloromethane (1:5:94 v/v/v).

5'-O-Dimethoxytrityl-N²-dimethylaminomethylidene-2'-O-allylguanosine-3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (VIII). Compound VII (4.94 g, 7.25 mmol) was dried by evaporation of acetonitrile (20 ml) *in vacuo*. The foam was dissolved in dry 1,2-dichloroethane (30 ml) containing N,N-diisopropylethylamine (2.5 ml, 14.5 mmol) under argon and the solution was cooled in an ice-bath. 2-Cyanoethoxy N,N-diisopropylaminochlorophosphine (2.41 ml, 10.88 mmol) was added dropwise with stirring during 2 min. The reaction was kept 5 min at 0°C and was then stirred for 1 h at room temperature. T.l.c. in dichloromethane/ethanol/triethylamine (98:10:2 v/v/v) showed complete reaction. The reaction was quenched by addition of ethanol (2 ml). After 2 min, dichloromethane (70 ml) was added and the solution was washed with 1 M aqueous sodium bicarbonate (100 ml) followed by saturated brine (100 ml). The organic layer was dried (Na_2SO_4), filtered, and solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (120 g) eluting with a gradient of ethanol from 0 to 2% in dichloromethane/triethylamine (98:2 v/v). Pure title compound was obtained as a solid white foam (5.95 g, 93%) after lyophilisation from benzene, with R_f 0.66 on t.l.c. in dichloromethane/ethanol/triethylamine (98:10:2 v/v/v). ^{31}P n.m.r. spectrum (CH_2Cl_2, external concentric D_2O lock) δ: + 147.19 and 147.02 ppm.

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-N²,N⁶-bis(phenoxyacetyl)-2'-O-allyl-2,6-diaminopurine riboside (IX). Compound IV (11.4 g, 20 mmol) was dissolved in dry pyridine (100 ml) and stirred with phenoxyacetic anhydride (28.6 g, 100 mmol) under dry nitrogen. T.l.c. in petrol/ethyl acetate (1:1 v/v) after 4 h showed complete reaction with a new spot at R_f 0.59. Water (10 ml) was added and the mixture was left stirring for 3 h to quench excess anhydride. Solvent was then removed *in vacuo* and the residue dissolved in ethyl acetate (1 l). The solution was washed with 1 M aqueous sodium bicarbonate (1 l), dried (Na_2SO_4), filtered and solvent removed *in vacuo*. The crude product was purified by preparative liquid chromatography on a single silica cartridge using petrol/ethyl acetate (3:2 v/v) as eluant. The title compound was obtained as a solid white foam (13.9 g, 82.9%) of R_f 0.59 on t.l.c. in petrol/ethyl acetate (1:1 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 167.28 and 166.46 (C=Os of phenoxyacetyls), 157.20 (C-1s of phenyls), 151.49 and 151.28 (C-2 and C-6), 148.46 (C-4), 140.76 (C-8), 134.34 (allyl CH), 129.68 (C-3s and C-5s of phenyls), 122.15 (C-4s of phenyls), 120.08 (C-5), 117.33 (allyl $=CH_2$), 114.99 and 114.89 (C-2s and C-6s of phenyls), 88.52 (C-1'), 81.71 (C-2'), 80.98 (C-4'), 71.62 (allyl CH_2O), 68.94 (C-3'), 68.39 and 68.09 (CH_2s of phenoxyacetyls), 59.78 (C-5'), 17.44–16.87 (isopropyl CH_3s), 13.43, 12.90 and 12.60 p.p.m. (isopropyl CHs).

N²,N⁶-Bis(phenoxyacetyl)-2'-O-allyl-2,6-diaminopurine riboside (X). Compound IX (13.9 g, 16.65 mmol) was desilylated as described for the preparation of compound V. The reaction was quenched after 4 min and worked up as described. The crude product was purified by column chromatography on silica gel (300 g) eluting with a gradient from 0 to 5% ethanol in chloroform. The title compound was obtained as a solid white foam (7.88 g, 79.5%) of R_f 0.21 on t.l.c. in ethanol/dichloromethane (5:95 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 168.17 and 166.80 (C=Os of phenoxyacetyls), 157.18 and 156.95 (C-1s of phenyls), 151.39 and 150.86 (C-2 and C-6), 148.58 (C-4), 142.95 (C-8), 133.18 (allyl CH), 129.40 and 129.34 (C-3s and C-5s of phenyls), 121.83 and 121.60 (C-4s of phenyls), 119.39 (C-5), 118.06 (allyl $=CH_2$) 114.65 (C-2s and C-6s of phenyls), 87.93 (C-1'), 86.48 (C-4'), 80.67 (C-2'), 71.58 (allyl CH_2O), 69.79 (C-3'), 68.28 and 67.70 (CH_2s of phenoxyacetyls), 61.84 ppm (C-5').

5'-O-Dimethoxytrityl-N²,N⁶-bis(phenoxyacetyl)-2'-O-allyl-2,6-diaminopurine riboside (XI). Compound X (7.88 g, 13.23 mmol) was dimethoxytritylated according to the procedure used to prepare compound VII. The crude product was purified by preparative liquid chromatography on a single silica cartridge using petrol/ethyl acetate (2:1 v/v) containing 1% triethylamine as eluant. Pure title compound was obtained as a solid white foam (9.05 g, 76.2%) of R_f 0.29 in ethyl acetate/dichloromethane (1:2 v/v) containing 1% triethylamine.

5'-O-Dimethoxytrityl-N²,N⁶-bis(phenoxyacetyl)-2'-O-allyl-2,6-diaminopurine riboside-3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidite) (XII). Compound XI (9.05 g, 10.08 mmol) was phosphitylated as described for the synthesis of compound VIII. The crude product was purified by column chromatography on silica gel (200 g) using petrol/dichloromethane/THF/triethylamine (6:1:2:1 by vol.) as eluant. Pure title compound was obtained as a white solid (9.7 g, 87.6%) of R_f 0.60 and 0.52 in petrol/dichloromethane/triethylamine (6:3:1 v/v/v). ^{31}P n.m.r. spectrum (CH_2Cl_2, concentric external D_2O lock) δ: + 147.34 and 146.85 ppm.

RESULTS AND DISCUSSION

Our route to the 2'-O-allylguanosine building block (VIII) is illustrated in Figure 1, starting with the previously prepared key intermediate I (2). The recently described procedure for the allylation of alcohols under neutral conditions (4) was thought to be suitable for the rather acidic 2'-hydroxyl group of an appropriately protected ribonucleoside. This indeed proved to be the case; allylation of compound I to give compound II proceeded in 89% yield in 30 min using allyl ethyl carbonate in the presence of 0.5 mole% of tris(dibenzylideneacetone)dipalladium(0) and 2 mole% of 1,4-bis(diphenylphosphino)butane in refluxing tetrahydrofuran. This procedure is superior in terms of yield and cost to that using allyl bromide and BDDDP. Subsequent reaction of compound II with sodium azide in DMF afforded the 2,6-diazido purine compound III in 78% yield. Reduction to the 2'-O-allyl-2,6-diaminopurine riboside derivative IV proved to be rather difficult. This was initially performed with hydrogen in the presence of Lindlar catalyst further poisoned with quinoline. However, about 10% reduction of allyl to propyl was also observed. In fact all subsequent reductions employing Lindlar catalyst resulted in complete conversion of allyl to propyl. In

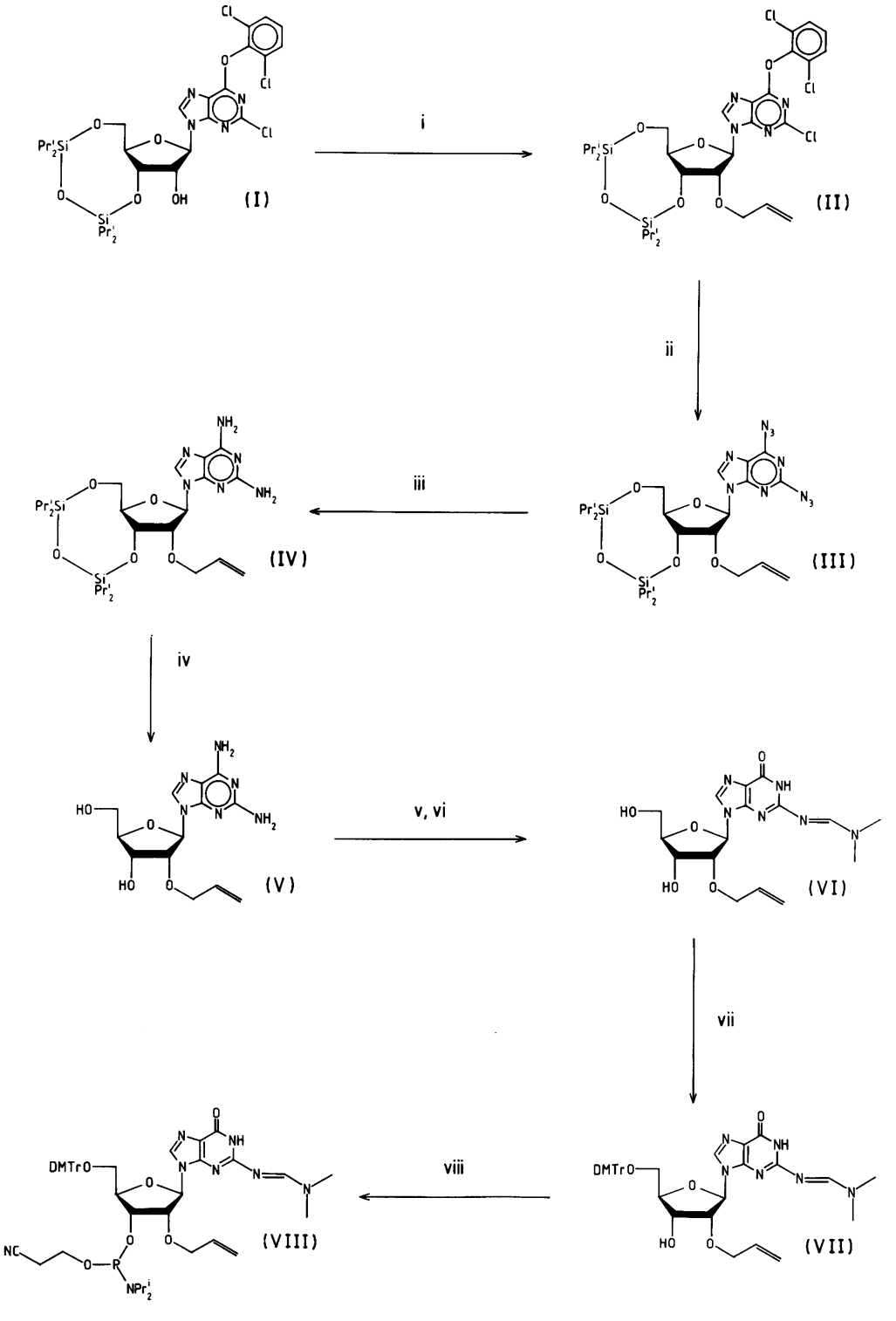

Figure 1. Reaction scheme for the preparation of the 2'-O-allylguanosine building block. Reagents: i, allyl ethyl carbonate, 1,4-bis(diphenylphosphino)butane and tris(dibenzylideneacetone)dipalladium(0) in tetrahydrofuran; ii, sodium azide in N,N-dimethylformamide; iii, stannous chloride, thiophenol and triethylamine in acetonitrile; iv, tetrabutylammonium fluoride in tetrahydrofuran; v, adenosine deaminase in aqueous phosphate buffer pH 7.4/dimethylsulphoxide; vi, N,N-dimethylformamide dimethyl acetal in methanol; vii, 4,4'-dimethoxytrityl chloride and triethylamine in pyridine; viii, 2-cyanoethoxy N,N-diisopropylaminochlorophosphine and N,N-diisopropylethylamine in 1,2-dichloroethane.

a search for alternative highly selective reducing agents for azide functions we found that the $[Sn(SPh)_3]^-$ ion was recently reported in the literature as being the best reducing agent for azides to amines (5, 6). Thus, using 3 equivalents of anhydrous stannous chloride, 12 equivalents of thiophenol and 9 equivalents of triethylamine in acetonitrile gave an absolutely clean reduction of compound III to compound IV in 93% yield in 15 min at room temperature. Subsequent desilylation afforded 2'-O-allyl-2,6-diaminopurine riboside, V, in 96% yield. Compound V is a reasonable substrate for adenosine deaminase, which had been

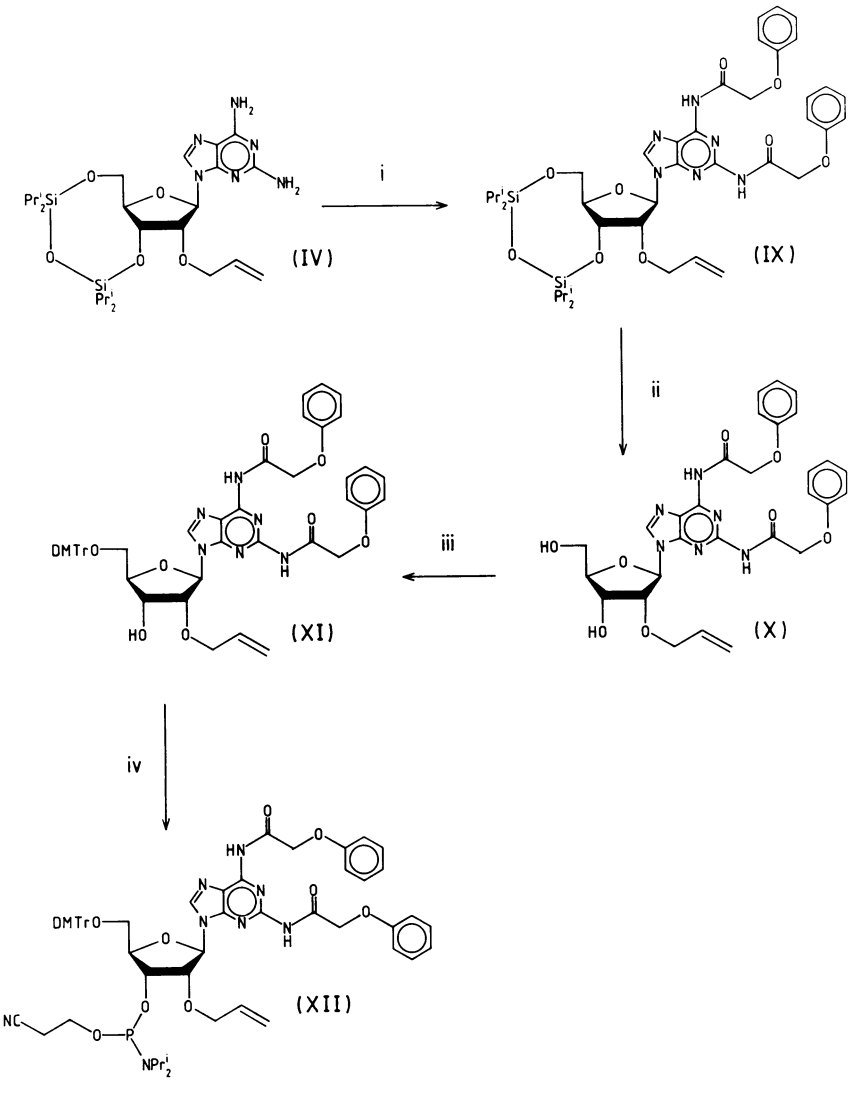

Figure 2. Reaction scheme for the synthesis of the 2′-O-allyl-2,6-diaminopurine riboside building block. Reagents: i, phenoxyacetic anhydride in pyridine; ii, tetrabutylammonium fluoride in tetrahydrofuran; iii, 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; iv, 2-cyanoethoxy N,N-diisopropylaminochlorophosphine and N,N-diisopropylethylamine in 1,2-dichloroethane.

used previously for converting 2′-O-methyl-2,6-diaminopurine riboside to 2′-O-methylguanosine (7). Quantitative reaction took 72 h at 37°C in phosphate buffer/DMSO and the 2-amino group of the resultant guanosine was protected directly using N,N-dimethylformamide dimethyl acetal (8), giving compound VI in 99% yield. Subsequent dimethoxytritylation and phosphitylation (9) afforded the desired synthon VIII. The overall yield of 2′-O-allylguanosine building block was 30% for the 13 step synthesis from 2-amino-6-chloropurine riboside.

Figure 2 illustrates the synthesis of the novel 2′-O-allyl-2,6-diaminopurine riboside building block XII, starting from the versatile intermediate IV. 2-Amino-2′-deoxyadenosine occurs naturally instead of 2′-deoxyadenosine in the DNA of the S-2L cyanophage (10) and can form three hydrogen bonds in a Watson–Crick 2-amino A·T base pair (11). Several groups have synthesized oligodeoxyribonucleotides containing 2-aminoadenine (12, 13) however, a variety of problems exist. The main one is the depurination of acid-labile di-N-acyl derivatives of 2-amino-2′-deoxyadenosine (12), which can be overcome using an amidine protecting group for the 6-amino group (13). A second problem is the requirement of stringent deprotection conditions (13). To avoid depurination in another way Kikuchi *et al.* (14) incorporated 2-aminoadenosines in synthetic oligodeoxyribonucleotides using N-acetyl protection. A reason for this was that elevation of the T_m value by the 2-amino group is larger in the ribo than in the deoxyribo series (15). It was clear that 2′-O-allyl-oligoribonucleotides containing 2,6-diaminopurine should be useful probes for selecting ribonucleic acid-protein complexes in which only short stretches of RNA are available for hybridisation. Moreover, in the ribo series depurination of di-N-acyl derivatives of 2-aminoadenine is expected to be negligible. In order to protect the amino groups of compound IV we chose to use the base labile phenoxyacetyl group developed by Teoule *et al.* (16) for protection of dA and dG and used by Wu *et al.* (17) for protection of rA and rG. Thus compound IV was treated with excess phenoxyacetic anhydride in pyridine followed by an aqueous pyridine work up to give compound IX in 83% yield. A subsequent 4 min desilylation afforded an 80% yield of N^2,N^6-bis(phenoxyacetyl)-2′-O-allyl-2,6-diaminopurine riboside, compound X. Subsequent dimethoxytritylation and phos-

phitylation yielded the desired building block XII. The overall yield of the 2ó-O-allyl-2,6-diaminopurine riboside building block, XII was 16% for the 12 step synthesis starting from 2-amino-6-chloropurine riboside.

Appropriately protected 2′-O-allyluridine and 2′-O-allylcytidine building blocks were prepared in overall yields of 28% and 34% respectively from uridine via allylation of 3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-4-O-(2,6-dichlorophenyl)uridine. The latter compound was synthesized analogous to the 4-O-(2-nitrophenyl) derivative as described by Nyilas and Chattopadhyaya (18). The intermediate 3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-2′-O-allyl-4-O-(2,6-dichlorophenyl)uridine treated with tetrabutylammonium fluoride followed by 2-nitrobenzaldoximate (19, 20) gave 2′-O-allyluridine, whereas ammonia treatment afforded siloxane protected 2′-O-allylcytidine.

The 2′-O-allyladenosine and 2′-O-allylinosine monomers were synthesized via palladium(0) catalysed allylation of the previously described intermediate, 3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-6-(2,6-dichlorophenoxy)purine riboside (2). The 2′-O-allyl A and I monomers were then obtained in analogous fashion to the 2′-O-methyl ones (2) except that for the inosine compound desilylation was performed prior to the oximate reaction. The overall yields of the 2′-O-allyl A and 2′-O-allyl I monomers were 34 and 20% respectively starting from 6-chloropurine riboside.

Oligo(2′-O-allylribonucleotides) were synthesized as described for the 2′-O-methyl analogues (21) using a coupling time of 8 min, and multiple biotinylation when required was performed during the solid phase synthesis (22). The 2′-O-allyl-2-aminoadenosine monomer, XII, was insoluble in pure acetonitrile and was dissolved in THF/acetonitrile (1:1 v/v) for synthesis. The capping reaction for oligomers containing 2′-O-allyl-2,6-diaminopurine riboside must be carried out with phenoxyacetic anhydride/N-methylimidazole instead of acetic anhydride to avoid a transamidation reaction (23). A short biotinylated 2′-O-allyloligoribonucleotide containing five 2-aminoadenines from a total of 12 hydrogen bonding bases has proved essential for the efficient depletion of human U5 snRNP from crude nuclear extracts (Lamm et al., manuscript in preparation).

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