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High Yield Protection of Purine Ribonucleosides for Phosphoramidite RNA Synthesis

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Abstract: We report high yield procedures for protection of purine ribonucleosides based on a reaction which allows concomitant highly regiospecific 2'-silylation and 3'-phosphitylation. Subsequent cleavage of the H-phosphonate monoester moiety without silyl migration provides intermediates ready for phosphitylation by standard methods to give fully protected phosphoramidites.
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We earlier reported efficient procedures for protection of ribonucleosides for RNA synthesis by the H-phosphonate method, based on a highly regiospecific silylation of the 2' hydroxyl group.[1] In an effort to extend this silylation to preparation of phosphoramidites, we have now developed a facile method for quantitative cleavage of the H-phosphonate group, without silyl migration. Moreover, we also report a new reaction that allows one-step silylation and phosphitylation with the same high specificity as the previous method.

The procedures reported below for adenosine[2] and guanosine[3] differ only in the order of introduction of the phenoxyacetyl amino protecting group.[4-7] Despite the significant progress on use of amino-unprotected monomers in phosphoramidite synthesis,[8-10] complications remain, and we chose to use amino protection. With both nucleosides we use transient protection of the 2', 3' diol moiety of the ribonucleosides by reaction with *N,N*-dimethylformamide dimethylacetal[11] to prevent the small, but potentially troublesome, tritylation of the 2'-hydroxyl that otherwise accompanies tritylation of the 5'-hydroxyl.[1]

Reaction of adenosine (**1**) with *N,N*-dimethylformamide dimethylacetal gives quantitative conversion to **2**, where both the 2', 3' diol and the amino group are protected, leaving only the 5'-hydroxyl free for tritylation. The 2',3'-*O*-dimethylaminomethylene moiety is cleaved by any protic solvent, while the *N*-dimethylamino-methylene is cleaved by treatment with either aqueous ammonia or methylamine. Transient protection of **3** with trimethylchlorosilane followed by acylation using the hydroxybenzotriazole active ester of phenoxyacetic acid[5,6] gives **4** in an overall yield, from adenosine, of over 90%. The acylation of guanosine (**5**) is carried out first to give the *N*-acyl derivative **6** as crystalline material in 95% yield. Reaction with *N,N*-dimethylformamide dimethylacetal to protect the 2', 3' diol followed by tritylation gives **8** in an overall yield from **5** of 89%.

We had shown previously that, for adenosine and guanosine, reaction of a mixture (~1:1) of 2'(3') H-phosphonates with *tert*butyldimethylchlorosilane and DBU proceeds with 85 - 90% selectivity in favor of the 2' silyl compound. We now report that silylation and phosphitylation can be carried out concomitantly by treatment of **4/8** with a mixture of phenyl-H-phosphonate, BDMS-Cl, and DBU. Presumably, the phenyl-H-phosphonate reacts first with *tert*-butyldimethylchlorosilane to generate the diester, which then undergoes a transesterification with **4/8** to generate a mixture of isomers (**9**), analogous to that produced by silylation of a mixture of the corresponding 2'(3') H-phosphonates. Transfer of the BDMS group predominantly to the more acidic 2'-hydroxyl gives **10** along with 10-15% of the 3'-*O*- BDMS isomer (not shown). The isomers are readily separated by chromatography on silica gel, but the polar conditions required by the H-phosphonate tend to cause some loss of the labile phenoxyacetyl group. Instead, it is better to use crude **10** in the next step and separate the isomers at the end. In order to remove the H-phosphonate group from **10** so that it can be converted to a

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adenosine (1) → $(CH_3)_2NCH(OCH_3)_2$ / pyridine → 2 (HO, A–N=CHN$(CH_3)_2$, H, N$(CH_3)_2$) → 1. DMTCl/pyr / 2. aq NH_3 → 3 (DMTO, ANH_2, HO, OH) → 1) TMS-Cl / 2) PhOAc-HOBT → 4 (DMTO, A-NHAcOPh, HO, OH)

guanosine (5) → 1) TMS-Cl / 2) PhOAc-HOBT → 6 (HO, G-NHAcOPh, HO, OH) → $(CH_3)_2NCH(OCH_3)_2$ / pyridine → 7 (HO, G-NHAcOPh, H, N$(CH_3)_2$) → 1. DMTCl/pyr / 2. CH_3OH → 8 (DMTO, G-NHAcOPh, HO, OH)

4/8 (DMTO, B, HO, OH) → $PhOPHO_2^-$ / BDMS-Cl / DBU → [9 (DMTO, B, O, OH, H–P–O–Si, O ⇌ DMTO, B, HO, O, Si–O–P–H, O)] → 10 (DMTO, B, O, OBDMS, H–P–O^-, O)

10 → Ad-Cl, glycerol (HO, OH, HO) → [11 (DMTO, B, O, OBDMS, H, P, O, ^-O, O, OH)] → 12 (DMTO, B, HO, OBDMS)

a; B = NH-AcOPh-adenine
b; B = NH-AcOPh-guanine

phosphoramidite, we make use of the extraordinarily facile transesterification of H-phosphonate diesters in the presence of a vicinal hydroxyl group. Standard H-phosphonate coupling of **10** with ethylene glycol or glycerol along with either pivaloyl or adamantoyl chloride effects the conversion to **12** quantitatively within minutes, presumably via the intermediate **11**. Silica gel chromatography then gives pure **12** in overall yields of about 65% from **4/8** or about 60% from **1** or **5**. This is more than three times the best overall yield that is possible with conventional procedures.

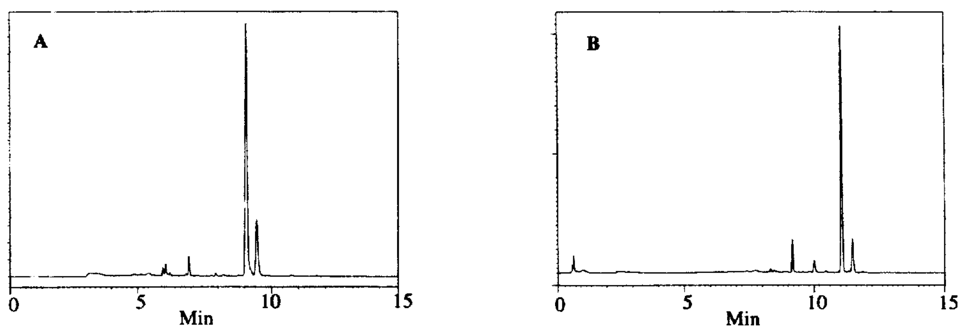

Figure 1. Reversed phase HPLC profiles (2-80% acetonitrile: 0.1 M triethylammonium acetate in 5 min at 2 mL/min on a Waters 3.9x150 mm Nova-Pak® C18 column) of the crude mixture of **12** (main peak, 85-90%) and the 3'-silyl isomer (longer retention peak) for: panel A, adenosine; panel B, guanosine.

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References and Notes

1. Zhang, X.; Abad, J.-L.; Huang, Q.; Zeng, F.; Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1997,** *38*, 7135-7138.
2. Synthesis of **12a:** To adenosine (5.34 g, 20 mmol), dried by co-evaporation with pyridine (2 x 100 mL) and suspended in 150 mL of dry pyridine, was added dimethylformamide dimethyl acetal (11.0 mL, 80 mmol). The mixture was stirred for 15 h and then concentrated. To the residue dissolved in dry pyridine (100 mL) was added dimethoxytrityl chloride (8.14 g, 24 mmol). The mixture was stirred for 3 hrs and then quenched with methanol (10 mL) and poured into saturated aq $NaHCO_3$ (150 mL). The mixture was extracted with dichloromethane (2 x 150 mL), and the combined organic layers were concentrated. To the residue dissolved in pyridine (100 mL) was added concentrated aq ammonia (100 mL). The solution was sealed and heated at 60 °C for 5 h. The mixture was then co-evaporated with pyridine to give **3**. To one half of crude **3** dissolved in dry pyridine (100 mL) was added N-methylmorpholine (11.0 mL, 100 mmol). This mixture was cooled in an ice-bath under nitrogen, and trimethylchlorosilane (5.06 mL, 40 mmol) was added over 5 min. This solution was stirred at room temperature for 2 h. The acylating reagent was prepared by co-evaporating 1-hydroxybenzotriazole (4.05 g, 30 mmol) and N-methyl-morpholine (4.4 mL, 40 mmol) with dry acetonitrile (2 x 20 mL), dissolving the mixture in dichloromethane (40 mL), adding phenoxyacetyl chloride (4.17 mL, 30 mmol), and shaking the mixture for 10 min. This reagent was then added to the nucleoside solution. The mixture was stirred for 12 h and then poured into saturated aq $NaHCO_3$ (150 mL). The product was extracted with dichloromethane (2 x 150 mL), and the combined organic layers were concentrated and dissolved in 100 mL of pyridine. Water (50 mL) was added and the mixture was stirred overnight. The solution was then concentrated, and the residue was purified by chromatography on silica gel using ethyl acetate:petroleum ether to give 6.42 g (9.1 mmol, 91% from **1**) of pure **4**. Ammonium phenyl-H-phosphonate[12] was prepared by adding 38.3 mL of diphenyl phosphite (0.20 mol) over 10 min to 400 mL of 7.4 M aq ammonia. This mixture was stirred for 1 h and then concentrated. The residue was co-

evaporated with absolute ethanol (2 x 100 mL) and triturated with ethyl ether (400 mL) for 30 minutes. The product was isolated as 29.9 g of a colorless crystalline solid (0.17 mol, 85%). A portion of ammonium phenyl-H-phosphonate (2.63 g, 15 mmol) was converted to the DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) salt by addition of DBU (2.3 mL, 15 mmol) and co-evaporation with pyridine (50 mL).The residue was dissolved in anhydrous pyridine (100 mL), and **4** (3.52 g, 5 mmol) was added, followed by BDMS-Cl (*tert*-butyldimethylsilyl chloride) (2.26 g, 15 mmol) and DBU (3.8 mL, 25 mmol). The mixture was stirred for 48 h and then poured into aq potassium phosphate (100 mL, 0.5 M, pH 7.0) and extracted with dichloromethane (2 x 100 mL). The combined organic layers were dried and concentrated. To a mixture of the residue and glycerol (1.38 g, 15 mmol), first dried by co-evaporation with pyridine (50 mL) and dissolved in dry pyridine (50 mL), was added 1-adamantanecarbonyl chloride (2.98 g, 15 mmol). After stirring 10 min, the solution was poured into aq potassium phosphate (100 mL) and extracted with dichloromethane (2 x 100 mL). The combined organic layers were dried and concentrated. The residue was purified by chromatography on silica gel using petroleum ether:ethyl acetate (3:2) to give 2.65 g of **12a** (3.24 mmol, 65% from **4**, 59% from **1**).

3. Synthesis of **12b:** To guanosine (5.66 g, 20 mmol) dried by co-evaporation with pyridine (2 x 100 mL) and suspended in anhydrous pyridine (200 mL) was added over 5 min trimethylchlorosilane (15.2 mL, 120 mmol). This solution was stirred for 1 h. The acylating reagent was prepared by co-evaporating 1-hydroxybenzotriazole (5.40 g, 40 mmol) and N-methylmorpholine (4.40 mL, 40 mmol) with dry acetonitrile (2 x 50 mL), dissolving the mixture in dichloromethane (100 mL), adding phenoxyacetyl chloride (5.53 mL, 40 mmol) to it, and shaking the mixture for 10 min. This reagent was then added to the nucleoside solution. After 36 h, water (50 mL) was added, the mixture was concentrated, and the residue was co-evaporated with water (2 x 50 mL). The crystalline solid that formed during concentration was shaken with water (50 mL), filtered and washed with water (20 mL) and 2-propanol (3 x 20 mL) to give 7.90 g of **6** as a colorless solid (18.9 mmol, 95%). To a portion of **6** (4.17 g, 10 mmol) dissolved in anhydrous pyridine (50 mL) was added dimethylformamide dimethyl acetal (1.59 mL, 11.6 mmol). After 1 h, the solution was concentrated to a gum. To the residue dissolved in dry pyridine (50 mL) was added dimethoxytrityl chloride (4.07 g, 12 mmol). The mixture was stirred for 2 h and then poured into aq potassium phosphate (100 mL, 0.5 M, pH 7.0). The mixture was extracted with dichloromethane (2 x 100 mL). The combined organic layers were dried and concentrated. The residue was dissolved in dichloromethane (10 mL) and precipitated by addition to diethyl ether. Filtration of the precipitate gave 7.3 g of crude **8** (est. 94% pure). To 3.7 g of crude **8** dissolved in anhydrous pyridine (100 mL) was added ammonium phenyl-H-phosphonate (2.63 g, 15 mmol), prepared as above, and DBU (2.3 mL, 15 mmol), that had been co-evaporated with pyridine (50 mL), followed by BDMS chloride (2.26 g, 15 mmol) and DBU (3.8 mL, 25 mmol). The mixture was stirred for 48 h and then poured into aq potassium phosphate (100 mL, 0.5 M, pH 7.0) and extracted with dichloromethane (2 x 100 mL). The combined organic layers were dried and concentrated. To the residue and glycerol (1.38 g, 15 mmol), dried by co-evaporation with pyridine (50 mL) and dissolved in dry pyridine (50 mL), was added 1-adamantanecarbonyl chloride (2.98g, 15 mmol). After 10 min of stirring, the solution was poured into aq potassium phosphate (100 mL) and extracted with dichloromethane (2 x 100 mL). The combined organic layers were dried and concentrated. The residue was purified by chromatography on silica gel using acetone:dichloromethane (5:95 to 15:85), to give 2.63 g of **12b** (3.15 mmol, 63% from **5**).

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