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Preparation of Formacetal-Linked Purine-Purine Dinucleotide Analogs

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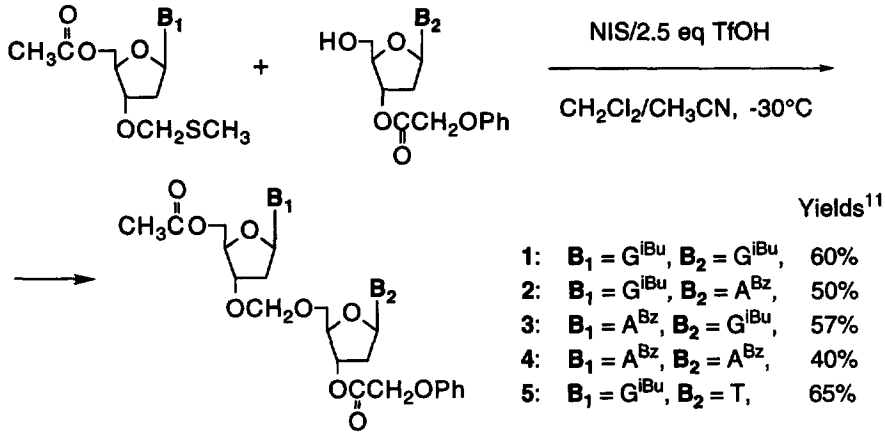
Abstract: Protected formacetal-linked purine-purine dinucleotide analogs, including dG-f-dG, dG-f-dA, dA-f-dG, and dA-f-dA, were synthesized for the first time in 40 - 60% yields by condensation of the 5'-OH group with the 3'-OCH_2SCH_3 group of the two corresponding deoxynucleoside units using *N*-iodosuccinimide in the presence of 2.5 eq of trifluoromethanesulfonic acid at -30°C.

Numerous efforts have been devoted to the synthesis of oligonucleotide analogs with modified backbones because of their interesting physicochemical and biological properties.[1,2] Particularly, replacement of the phosphate diester linkage with a neutral surrogate has been an area of interest in order to improve the permeation efficiency of antisense agents.[3,4] The formacetal linkage is especially interesting because it is achiral and incorporation of the formacetal linkage into oligonucleotides was found to cause minimal structural perturbations.[5]

While the synthesis of formacetal-linked dinucleotide analogs containing a pyrimidine has been described, the purine-purine analog has not been reported to date. Matteucci et al. reported a method of using activation of the 3'-O-CH_2SCH_3 (MTM) of one nucleoside with *N*-bromosuccinimide (NBS) or bromine in the presence of the 5'-OH of the other nucleoside to prepare the formacetal linkage between two thymidine units.[6,7] van Boom et al. used a condensation of the 3'-O-CH_2-$OP(O)(OR)_2$ with the 5'-OH in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the synthesis of formacetal-linked pyrimidine-pyrimidine or pyrimidine-purine dinucleotide analogs.[8,9] As part of a program investigating the structure-activity relationship of an oligodeoxynucleotide thrombin inhibitor,[10] we required two 2'-deoxyguanosine units linked through a formacetal linkage (dG-f-dG). However, attempts to synthesize dG-f-dG by existing methods were unsatisfactory. Condensation of 3'-O-MTM with 5'-OH using NBS or bromine activation resulted in a complex mixture. Under van Boom's conditions using catalytic amounts of TMSOTf, no reaction occurred, while excess TMSOTf (2 - 4 eq) resulted primarily in silylation of the 5'-OH at low temperatures (-30 °C) or in extensive decomposition at higher temperatures (0°C). The problems in the condensation of two dG units could be caused by the nucleophilic nitrogen atoms of



the nucleoside bases. This could explain the difficulties for the synthesis of formacetal-linked purine-purine dinucleotide analogs through a carbocation intermediate.

In an earlier study, van Boom et al. synthesized the formacetal linkage between two thymidine units by activating the 3'-O-MTM with *N*-iodosuccinimide (NIS) in the presence of a catalytic amount of trifluoromethanesulfonic acid (TfOH) at 0°C.[12] However, extending the reaction conditions to the preparation of formacetal-linked dinucleotide analogs containing other nucleosides, including dC, dG, and dA, resulted in no desired product.[8,9,13] We have found that by conducting the reaction with 2.5 eq of TfOH, in order to minimize the effect of the nucleophilic base nitrogen atoms, under anhydrous conditions at low temperature where the purine nucleotide is stable to the acid, dG-f-dG can be prepared in 60% yield. In a representative experiment, N^2-isobutyryl-3'-O-methylthiomethyl-5'-O-acetyl-2'-deoxyguanosine[14] (100 mg, 1 eq) and N^2-isobutyryl-3'-O-phenoxyacetyl-2'-deoxyguanosine (100 mg, 1 eq) were dissolved in CH_2Cl_2 (2 mL) and CH_3CN (2 mL). The solution was dried over 4Å molecular sieves (0.2 g of 8-12 mesh beads) for 1 hour and cooled to -30°C. TfOH (75 mg, 2.5 eq) was added dropwise followed immediately by NIS (60 mg, 1.2 eq). The mixture was stirred at -30°C for 1 hour. The NIS powder completely dissolved and the solution turned to dark brown. Triethylamine (100 mg, 10 eq) was added slowly at -30°C to neutralize the acid, and the resulting mixture was poured into 100 mL of 5% aqueous $Na_2S_2O_3$ solution. The mixture was extracted (CH_2Cl_2), and the organic phase was dried (Na_2SO_4). After the solvent was removed, the solid residue was purified by column chromatography (SiO_2/CH_2Cl_2:EtOAc:MeOH = 50:50:8) to give **1**.[15]

These reaction conditions were also used to prepare the protected formacetal-linked dinucleotide analogs dG-f-dA (**2**),[16] dA-f-dG (**3**),[17] dA-f-dA (**4**),[18] and dG-f-T (**5**),[19] respectively, in the yields listed above (1.5 eq of TfOH was used in the

preparation of **5**).[11] Using 2.5 eq of methanesulfonic acid gave similar results although the yields were relatively low (20 - 30%), while dibenzyl phosphate resulted in decomposition of the 3'-O-MTM-nucleoside. When using CH_2Cl_2 as the sole solvent, the adddition of TfOH to the reaction mixture at -30°C generated an oily precipitate, resulting in a low yield (10%). A certain amount of depurination products was formed when the reaction temperature exceeded -10°C, and the reaction was slow when the temperature was lower than -50°C. In addition to the desired product, the crude product mixture contained mainly unreacted 5'-OH-nucleoside and hydrolyzed 3'-O-MTM-nucleoside (20 - 30%). Therefore, hydrolysis of the 3'-O-MTM group is the major side reaction, which provides some room for future improvement.

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15. **1**: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 12.07, 12.06. 11.66, 11.62(4s, NH, 4H), 8.24, 8.15(2s, 8-H, 2H), 7.3 - 6.9(m, Ar-H, 5H), 6.23, 6.14(2t, J = 6.5, 7 Hz, 1'-H, 2H), 5.43(m, 3'-H, 1H), 4.81(s, -$COCH_2OPh$, 2H), 4.76(dd, AB, -OCH_2O- J = 7 Hz, 2H), 4.43(m, 3'-H, 1H), 4.24(m, 4'-H, 1H), 4.09(m, 4'-H, 5' and 5"-H, 3H), 3.73(m, 5' and 5"-H, 2H), 2.99(m, 2"-H, 1H), 2.8-2.6(m, 2"-H, 2 x -CH-, 3H), 2.55(m, 2'-H, 1H), 2.46(m, 2'-H, 1H), 1.98(s, -$COCH_3$, 3H), 1.10(d, J = 7 Hz, 12H). The formation of 3'-O-CH_2-O-5' linkage was further confirmed by 2D NMR techniques, including COSY, HMQC and HMBC (C-H single-bond and multi-bond correlation).
16. **2**: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 12.05, 11.61, 11.20(3s, NH, 3H), 8.76, 8.68, 8.16 (3s, Ar-H, 3H), 8.1 - 6.9(m, Ar-H, 10H), 6.51, 6.13(2t, J = 7, 6.5 Hz, 1'-H, 2H), 5.52(m, 3'-H, 1H), 4.88(s, -$COCH_2OPh$, 2H), 4.75(dd, AB, -OCH_2O-, J = 5 Hz, 2H), 4.40(m, 3'-H, 1H), 4.31(m, 4'-H, 1H), 4.18(m, 4'-H, 1H), 4.11(s, 5' and 5"-H, 2H), 3.78(m, 5' and 5"-H, 2H), 3.20(m, 2"-H, 1H), 2.8-2.6(m, 2"-H, 2'-H, 2 x -CH-, 4H), 2.41(m, 2'-H, 1H), 1.94(s, -$COCH_3$, 3H), 1.10(d, J = 6.5 Hz, 6H).
17. **3**: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 12.0, 11.6, 11.2(3bs, NH, 3H), 8.73, 8.61, 8.27 (3s, Ar-H, 3H), 8.1 - 6.9(m, Ar-H, 10H), 6.43, 6.24(2t, J = 6.5, 6 Hz, 1'-H, 2H), 5.46(m, 3'-H, 1H), 4.85(s, -$COCH_2OPh$, 2H), 4.78(dd, AB, -OCH_2O-, J = 5 Hz, 2H), 4.56(m, 3'-H, 1H), 4.25(m, 4'-H, 1H), 4.18(m, 4'-H, 5' and 5"-H, 3H), 3.77(m, 5' and 5"-H, 2H), 3.0-2.9(m, 2 x 2"-H,2H), 2.88(m, 2'-H, 1H), 2.75(m, 2 x -CH-, 2H), 2.55(m, 2'-H,1H), 1.94(s, -$COCH_3$, 3H), 1.11(d, J = 6.5 Hz, 6H).
18. **4**: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.20(s, NH, 2H), 8.75, 8.72, 8.69, 8.61(4s, Ar-H, 4H), 8.1 - 6.9(m, Ar-H, 5H), 6.51, 6.43(2t, J = 7, 6.5 Hz, 1'-H, 2H), 5.56(m, 3'-H, 1H), 4.88(s, -$COCH_2OPh$, 2H), 4.79(dd, AB, -OCH_2O-, J = 6 Hz, 2H), 4.55(m, 3'-H, 1H), 4.34(m, 4'-H, 1H), 4.2-4.0(m, 4'-H, 5' and 5"-H, 3H), 3.82(m, 5' and 5"-H, 2H), 3.22(m, 2"-H, 1H), 2.97(m, 2"-H, 1H), 2.63(m, 2'-H, 1H), 2.56(m, 2'-H,1H), 1.92(s, -$COCH_3$, 3H).
19. **5**: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 12.07, 11.61, 11.36(3s, NH, 3H), 8.20 (s, 8-H, 1H), 7.54(s, 6-H, 1H), 7.3 - 6.9(m, Ar-H, 5H), 6.19(m, 2 x 1'-H, 2H), 5.30(m, 3'-H, 1H), 4.84(s, -$COCH_2OPh$, 2H), 4.82(dd, AB, -OCH_2O-, J = 7.5 Hz, 2H), 4.48(m, 3'-H, 1H), 4.3-4.1(m, 2 x 4'-H, 5' and 5"-H, 4H), 3.77(m, 5' and 5"-H, 2H), 2.8-2.6(m, 2"-H, -CH-, 2H), 2.55(m, 2"-H, 1H), 2.40(m, 2'-H,1H), 2.30(m, 2'-H, 1H), 1.96(s, -$COCH_3$, 3H), 1.76(s, -CH_3, 3H), 1.10(d, J = 7 Hz, 6H).

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