Synthesis of 2′-Deoxyguanosine Containing a N^2-Polyamine

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Dedicated to the memory of Dr. Roland K. Robins

The synthesis of benzylated N^2-(4,7,10,13-tetraazatridec-1-yl)-2′-deoxyguanosines **4** was accomplished by a key nucleophilic reaction of the novel unsymmetrical polyamine **2**, with 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2-chloro-2′-deoxyinosine (**1**).

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Introduction.

Nucleosides have for some time played a major role in medicine as antiviral and antitumor chemotherapeutic agents [1-2]. Recently, considerable interest has been shown in the incorporation of unnatural nucleosides into oligonucleotides to enhance their pharmacokinetic and pharmacodynamic properties [3].

Our interest in examining novel nucleoside analogs for antiviral and antitumor activities as well as their subsequent incorporation into oligonucleotides prompted us to focus on the synthesis of N^2-purine modified nucleosides. Only a few synthetic efforts have been directed to attaching pendent moieties to the N^2-position of purines [4-7]. Niiya *et al.* prepared a variety of N^2-substituted purines for adenosine receptor properties [4]. N^2-Substituted guanine nucleosides and nucleotides have been reported to be selective inhibitors of DNA polymerases, thymidine kinases and G-proteins [5]. Also reported from Wright's Laboratory [6] was the synthesis of a series of N^2-phenylguanines that were examined as inhibitors of herpes simplex virus thymidine kinases. The N^2-position has also been utilized to attach polycyclic aromatic hydrocarbons moieties [7]. Furthermore, we have shown that N^2-imidazolylpropyl modified guanine and 2-aminoadenine in oligonucleotides accommodate the pendent group in the minor groove formed on hybridization of the modified oligonucleotides to complementary RNA or DNA [8].

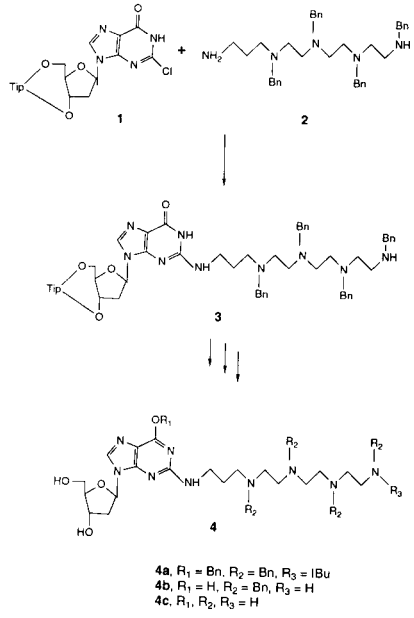
Our goal is to attach polyamine moieties to the N^2-position of purines primarily to examine their effects on the uptake of nucleosides and modified oligonucleotides into cells. Cellular uptake by passive diffusion and/or endocytotic pathways may be enhanced by polyamines and amphipathic polyamines [9]. We reasoned that substitution on an exocyclic position should have minimal effect on the electronic parameters of the purine ring system and that replacing just one hydrogen of the amino group would still allow Watson-Crick hydrogen bonding to complementary cytosine residues.

As an entry into the synthesis of novel compounds of this type, we elected to attach a benzylated unsymmetrical polyamine, 13-amino-1,4,7,10-tetra(phenylmethyl)-1,4,7,10-tetraazatridecane (**2**) at the C^2-position of guanine. The basicity of the tertiary amines are expected to have a range of several p*K*a units due to their electronic interactions [10-12]. The benzyl groups along with partial protonation of the amines under physiological conditions would provide an amphipathic pendent group: removal of the benzyl groups would yield a polyamine analog which may enhance the uptake of the deoxyguanosine conjugate by a receptor mediated transport system. In addition, deoxyguanosine polyamine **4a** was appropriately blocked for conversion into its 5′-dimethoxytrityl (DMT), 3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) derivative which would subsequently be used in automated synthesis.

Synthesis and Discussion.

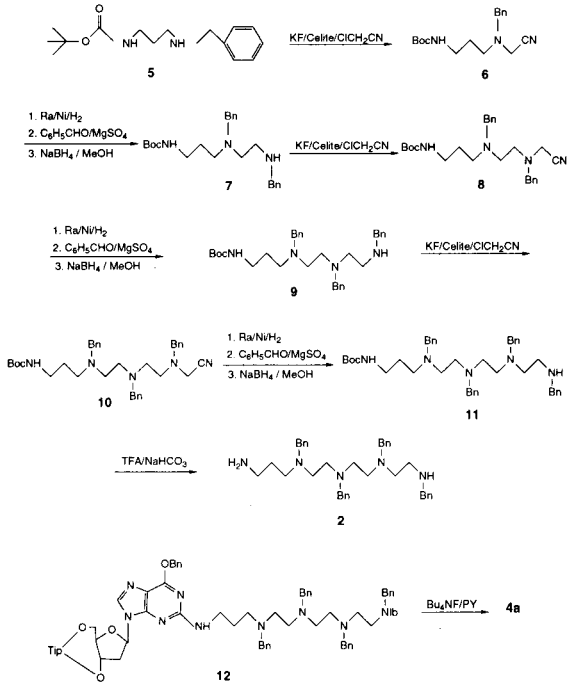
Our synthesis of the target nucleosides **4a-c** is shown in Scheme I. We envisioned that nucleophilic displacement of the chlorine atom in 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2-chloro-2′-deoxyinosine (**1**) with protected un-

Scheme I



symmetrical polyamine, 13-amino-1,4,7,10-tetra(phenylmethyl)-1,4,7,10-tetraazatridecane (**2**) should afford an intermediate **3** which on further chemical manipulation would provide the desired nucleoside polyamines **4a-c**. Synthesis of the nucleoside intermediate **1** was accomplished as reported earlier by us [8]. We next examined the preparation of the polyamine intermediate **2**. Starting material *N*-[*N*-(*tert*-butylcarbonyl)-3-aminopropyl]benzylamine **5** was prepared from commercially available *N*-(2-cyanoethyl)benzylamine by a literature procedure [13]. Alkylation of **5** with chloroacetonitrile in the presence of excess potassium fluoride-celite in acetonitrile [14] provided

Scheme II



the corresponding nitrile **6** as light yellow needles. This alkylation procedure was found to be convenient and performed in high yield under mild conditions. Reduction of the nitrile group in **6** was accomplished with Raney nickel in basic solution under hydrogen atmosphere [13] to provide the amine intermediate which was used without further purification (Scheme II).

Treatment of the above amine with benzaldehyde in the presence of anhydrous magnesium sulfate in methanol for 3 hours gave the corresponding imine, which, without isolation, was reduced with sodium borohydride to give the protected compound **7** in 60% yield. This process, (i) alkylation (potassium fluoride/celite-chloroacetonitrile), (ii) reduction (Raney/nickel), and (iii) reductive amination (benzaldehyde/sodium borohydride), was repeated two successive times starting with **7** to afford protected polyamine **11** in good yield. Exposure of **11** to trifluoroacetic acid followed by an alkaline wash provided the key intermediate **2**. Although a number of polyamine syntheses have been reported [15], relatively few syntheses of unsymmetrical polyamines are known. The present work describes the synthesis of a polyamine having three and two carbons between the nitrogens, an addition to this class of molecules.

Nucleoside polyamine **3** was obtained in 60% yield by heating inosine derivative **1** and protected polyamine **2** in 2-methoxyethanol [8]. Displacement of the chlorine atom preferentially occurred by the primary amine rather than by the secondary amine. Formation of the polyamine adduct through attachment of the secondary amine was not observed. Although primary and secondary amines are reactive nucleophiles, the primary amine in this case is relatively unhindered and thus more reactive than the secondary amine. The structure of **3** was confirmed by ^{1}H-nmr and elemental analysis. In the ^{1}H-nmr spectrum, an exchangeable proton appeared at δ 6.94, which is characteristic of an "NH" proton attached to the N^2-position of purine. The secondary "NH" proton having the benzyl blocking group appeared at δ 1.80. The proton signals further substantiate that only the primary amine of **2** displaces the chloro group of **1**. It is noteworthy that a 14-atom linear chain containing terminal primary and secondary amines can nucleophilically displace the chlorine atom in the deoxyinosine **1**. Polyamine conjugated nucleosides represents a new class of compounds.

After establishing the structure of nucleoside polyamine **3**, we focused our attention on the coversion of **3** to the target compounds **4a-c**. The isobutyryl derivative of **3** was subject to Mitsunobu reaction conditions [16] in the presence of benzyl alcohol provided protected nucleoside **12**. Removal of the 'TipSi' protecting group was achieved by treating **12** with tetrabutyl ammonium fluoride in a pyridine:tetrahydrofuran:water (5:4:1) mixture at room temperature [8] to afford partially protected deoxyguanosine polyamine, **4a**. This nucleoside is now appropriately protected for successive dimethoxytritylation and phosphitylation reactions required to afford a synthon for automated synthesis.

Nucleoside polyamine **3** was desilyated with conditions described above to provide the desired amphipathic nucleoside **4b**. Studies to debenzylate **4b** to afford the polyamine nucleoside **4c** are in progress.

In summary, the design and synthesis of 2′-deoxyguanosine containing a N^2-unsymmetrical polyamine which was subsequently modified to provide an intermediate for automated synthesis, **4a**, an amphipathic nucleoside **4b**, and a penultimate intermediate **4c** for the synthesis of a polyamine nucleoside was accomplished. The biological studies of this new class of compounds, deoxyguanosine polyamines, and oligonucleotides bearing the deoxyguanosine polyamines will be reported elsewhere.

EXPERIMENTAL

All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were recorded on Varian EM-200 spectrometer as 10% w/v solution in deuteriochloroform using 1% tetramethylsilane (TMS) as the internal standard. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ.

N-[*N*-(*tert*-Butyloxycarbonyl)-3-aminopropyl]benzylamine (**5**).

A solution of *N*-(3-aminopropyl)benzylamine (38 g, 231.71 mmoles) in dry tetrahydrofuran (300 ml) was cooled to 5° in an ice-alcohol bath. To this cold stirred solution 2-[[(*tert*-butyoxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON) (56.58 g, 230 mmoles) in dry tetrahydrofuran (300 ml) was added slowly during a 6 hour period. After the addition of BOC-ON, the reaction mixture was stirred at room temperature under argon for an additional 6 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in ether (750 ml). The ether extract was washed with 5% sodium hydroxide solution (4 x 100 ml), dried over anhydrous sodium sulfate and concentrated to dryness. The residue was purified by flash column using dichloromethane → methanol gradient. The pure fractions were pooled together and evaporated to give 49.5 g (81%) of the product as oil; ^{1}H nmr (deuteriochloroform): δ 1.42 (s, 9H, *t*-Boc), 1.65 (m, 2H, $CH_2CH_2CH_2$), 2.70 (t, 2H, CH_2NHCH_2), 3.20 (m, 2H, BocNHCH_2), 3.78 (s, 2H, ArCH_2), 5.32 (br s, 1H, BocN*H*), 7.30 (m, 5H, Ar*H*).

10-Cyano-9-(phenylmethyl)-2,2-dimethyl-3-oxa-4-oxo-5,9-diazadecane (**6**).

To a stirred solution of **5** (24 g, 91 mmoles) in dry acetonitrile (500 ml) was added potassium fluoride/celite (50 g) and chloroacetonitrile (27.3 g, 364 mmoles) at room temperature. The reaction mixture was placed in a preheated oil bath at 85° and allowed to stir at that temperature under argon for 12 hours. The reaction mixture was cooled, filtered and washed with dichloromethane (100 ml). The combined filtrate was evaporated to dryness. The residue was dissolved in dichloromethane (500 ml) and washed with 5% sodium bicarbonate solution (100 ml), water (100 ml) and brine (100 ml). The organic extract was dried over anhydrous sodium sulfate and concentrated to give a solid. The solid was crystallized from dichloromethane/hexane to give 24 g (87%) of **6** as colorless needles, mp 70-73°; ^{1}H nmr (deuteriochloroform): δ 1.44 (s, 9H, *t*-Boc), 1.71 (m, 2H, $CH_2CH_2CH_2$), 2.67 (t, 2H, J = 6.4 Hz, CH_2NHCH_2), 3.23 (m, 2H, BocNHCH_2), 3.46 (s, 2H, CH_2CN), 3.65 (s, 2H, ArCH_2), 4.85 (br s, 1H, BocN*H*), 7.33 (s, 5H, Ar*H*).

Anal. Calcd. for $C_{17}H_{25}N_3O_2$: C, 67.29; H, 8.31; N, 13.85. Found: C, 67.34; H, 8.45; N, 13.85.

9,12-Di(phenylmethyl)-2,2-dimethyl-3-oxa-4-oxo-5,9,12-triazadodecane (**7**).

The nitrile **6** (34 g, 112.21 mmoles) was dissolved in ethanol (100 ml) and placed in a parr hydrogenation bottle. Sodium hydroxide (7 g) was dissolved in water (20 ml), mixed with ethanol (180 ml) and added into the parr bottle. Raney/NI (5 g, wet) was added and shaken in a parr apparatus over hydrogen (45 psi) for 12 hours. The catalyst was filtered, washed with 95% ethanol (100 ml). The combined filtrate was concentrated to 100 ml and cooled to 5° in an ice bath mixture. The cold solution was extraced with dichloromethane (3 x 200 ml). The combined extract dried over anhydrous sodium sulfate and evaporated to give 32 g (92%) of an oily product. The product was used as such for the next reaction; ^{1}H nmr (deuteriochloroform): δ 1.32 (br s, 2H, NH_2), 1.42 (s, 9H, *t*-Boc), 1.67 (m, 2H, $CH_2CH_2CH_2$), 2.48 (m, 4H, $CH_2CH_2NH_2$), 2.75 (t, 2H, J = 6.4 Hz, CH_2NHCH_2), 3.15 (m, 2H, BocNHCH_2), 3.55 (s, 2H, ArCH_2), 5.48 (br s, 1H, BocN*H*), 7.31 (m, 5H, Ar*H*).

The above amine (33 g, 107.5 mmoles) in dry methanol (100 ml) was mixed with anhydrous magnesium sulfate (30 g) and allowed to stir at room temperature under an argon atmosphere. To this stirred solution benzaldehyde (13.2 g, 125 mmoles) was added and the stirring was continued for 4 hours under argon. The reaction mixture was diluted with methanol (150 ml) and cooled to −5° in an ice salt bath. Solid sodium borohydride (30 g) was added in 1 g lots at a time during a 2 hour period, keeping the reaction temperature below 0°. After the addition of sodium borohydride, the reaction mixture was allowed to stir at room temperature overnight and filtered over celite. The filtrate was evaporated to dryness. The residue was partitioned between water (350 ml)/ether (500 ml) and extracted in ether. The ether extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified on a silica gel column using dichloromethane → methanol as eluent. The pure fractions were pooled together and evaporated to give 35 g (82%) of **7** as oil; ^{1}H nmr (deuteriochloroform): δ 1.42 (s, 9H, *t*-Boc), 1.65 (m, 2H, $CH_2CH_2CH_2$), 1.75 (br s, 1H, $ArCH_2NH$), 2.55 (m, 4H, CH_2CH_2), 2.70 (t, 2H, J = 6.4 Hz, CH_2NHCH_2), 3.15 (m, 2H, BocNHCH_2), 3.52 (s, 2H, ArCH_2), 3.72 (s, 2H, ArCH_2), 5.55 (br s, 1H, BocN*H*), 7.28 (m, 10H, Ar*H*).

Anal. Calcd. for $C_{24}H_{35}N_3O_2$: C, 72.51; H, 8.87; N, 10.57. Found: C, 72.39; H, 8.77; N, 10.72.

13-Cyano-9,12-di(phenylmethyl)-2,2-dimethyl-3-oxa-4-oxo-5,9,12-triazatridecane (**8**).

The title compound **8** was prepared from **7** by following the procedure used for the preparation of **6**. Materials used: Substrate **7** (4.55 g, 11.46 mmoles), chloroacetonitrile (2.6 g, 34.38 mmoles), potassium fluoride/celite (9.0 g) and dry acetonitrile (100 ml). The crude product was purified by flash chromatography over silica gel using dichloromethane → acetone as the eluent to give 4.8 g (96%); ^{1}H nmr (deuteriochloroform): δ 1.42 (s, 9H, *t*-Boc), 1.68 (m, 2H, $CH_2CH_2CH_2$), 2.52 (m, 4H, CH_2CH_2), 2.68 (t, 2H, J = 6.2 Hz, CH_2NHCH_2), 3.22 (m, 2H, BocNHCH_2), 3.36 (s, 2H, CNCH_2), 3.50 (s, 2H, ArCH_2), 3.62 (s, 2H, ArCH_2), 5.72 (br s, 1H, BocN*H*), 7.32 (m, 10H, Ar*H*).

Anal. Calcd. for $C_{26}H_{36}N_4O_2$: C, 71.52; H, 8.31; N, 12.83. Found: C, 71.17; H, 8.14; N, 12.82.

9,12,15-Tri(phenylmethyl)-2,2-dimethyl-3-oxa-4-oxo-5,9,12,15-tetraazapentadecane (**9**).

The title compound **9** was prepared from **8** by following two step procedure used for the preparation of **7**. Materials used in the first step: The substrate **8** (25 g, 57.34 mmoles), Raney/Ni (5 g), sodium hydroxide in ethanol (200 ml, 7 g of sodium hydroxide was dissolved in 20 ml of water and mixed with ethanol) and ethanol used to dissolve the substrate (100 ml). The crude product was extracted in dichloromethane which on evaporation gave 22 g (87%) of an oily product; ^{1}H nmr (deuteriochloroform): δ 1.40 (s, 9H, *t*-Boc), 1.50 (m, 4H, $CH_2CH_2CH_2$ and NH_2), 2.48 (m, 8H, 2 CH_2CH_2), 2.66 (t, 2H, J = 6.2 Hz, CH_2NHCH_2), 3.24 (m, 2H, BocNHCH_2), 3.50 (s, 2H, ArCH_2), 3.56 (s, 2H, ArCH_2), 5.48 (br s, 1H, BocN*H*), 7.28 (m, 10H, Ar*H*).

Materials used in the second step: Above amine (24.4 g, 55.33 mmoles), benzaldehyde (6.36 g, 60.00 mmoles), magnesium sulfate (20.0 g) and dry methanol (200 ml). The crude product was purified by flash chromatography over silica gel using dichloromethane → methanol as the eluent to give 20.0 g (68%) of **9** as an oil; ^{1}H nmr (deuteriochloroform): δ 1.40 (s, 9H, *t*-Boc), 1.52 (m, 2H, $CH_2CH_2CH_2$), 1.84 (br s, 1H, $ArCH_2NH$), 2.38 (t, 2H, J = 6.2 Hz, CH_2NHCH_2), 2.54 (m, 8H, 2 CH_2CH_2), 3.08 (m, 2H, $BocNHCH_2$), 3.42 (s, 2H, $ArCH_2$), 3.50 (s, 2H, $ArCH_2$), 3.65 (s, 2H, $ArCH_2$), 5.45 (br s, 1H, BocNH), 7.28 (m, 15H, ArH).

Anal. Calcd. for $C_{33}H_{46}N_4O_2$: C, 74.67; H, 8.74; N, 10.56. Found: C, 74.92; H, 8.39; N, 10.71.

16-Cyano-9,12,15-tri(phenylmethyl)-2,2-dimethyl-3-oxa-4-oxo-5,9,12,15-tetraazahexadecane (**10**).

The title compound **10** was prepared from **9** by following the procedure used for the preparation of **6**. Materals used: Substrate **9** (8.30 g, 15.66 mmoles), chloroacetonitrile (3.52 g, 46.98 mmoles), potassium fluoride/celite (10.0 g) and dry acetonitrile (150 ml). The crude product was purified by flash chromatography over silica gel using dichloromethane → ethyl acetate as the eluent to give 7.6 g (85%); ^{1}H nmr (deuteriochloroform): δ 1.42 (s, 9H, *t*-Boc), 1.60 (m, 2H, $CH_2CH_2CH_2$), 2.42 (t, 2H, J = 6.2 Hz, CH_2NHCH_2), 2.60 (m, 8H, 2 CH_2CH_2), 3.14 (m, 2H, $BocNHCH_2$), 3.38 (s, 2H, $CNCH_2$), 3.48 (s, 2H, $ArCH_2$), 3.54 (s, 2H, $ArCH_2$), 3.60 (s, 2H, $ArCH_2$), 5.42 (br s, 1H, BocNH), 7.26 (m, 15H, ArH).

Anal. Calcd. for $C_{35}H_{47}N_5O_2$: C, 73.77; H, 8.32; N, 12.29. Found: C, 73.69; H, 8.19; N, 12.31.

9,12,15,18-Tetra(phenylmethyl)-2,2-dimethyl-3-oxa-4-oxo-5,9,12,15,18-petaazaoctadecane (**11**).

The titled compound **11** was prepared from **10** by following a two step procedure used for the preparation of **7**. Materials used in the first step: The substrate **10** (7 g, 12.30 mmoles), Raney/Ni (2 g), sodium hydroxide in ethanol (160 ml, 3.5 g of sodium hydroxide was dissolved in 10 ml of water and mixed with ethanol) and ethanol used to dissolve the substrate (100 ml). The crude product was extracted in dichloromethane which on evaporation gave 5.6 g (79%) as oil; ^{1}H nmr (deuteriochloroform): δ 1.40 (s, 9H, *t*-Boc), 1.50 (m, 4H, $CH_2CH_2CH_2$ and NH_2), 2.48 (m, 12H, 3 CH_2CH_2), 2.66 (m, 2H, CH_2NHCH_2), 3.24 (m, 2H, $BocNHCH_2$), 3.50 (s, 2H, $ArCH_2$), 3.56 (s, 4H, 2 $ArCH_2$), 3.62 (s, 2H, $ArCH_2$), 5.48 (br s, 1H, BocNH), 7.28 (m, 15H, ArH).

Materials used in the second step: Above amine (21.2 g, 36.74 mmoles), benzaldehyde (4.24 g, 40.00 mmoles), magnesium sulfate (10.0 g), dry methanol (200 ml) and sodium borohydride (4.85 g, 128.45 mmoles). The crude product was purified by flash chromatography over silica gel using dichloromethane → methanol as the eluent to give 18.67 g (77%) of **11** as oil; ^{1}H nmr (deuteriochloroform): δ 1.40 (s, 9H, *t*-Boc), 1.52 (m, 2H, $CH_2CH_2CH_2$), 2.05 (br s, 1H, $ArCH_2NH$), 2.38 (t, 2H, J = 6.0 Hz, CH_2NHCH_2), 2.54 (m, 12H, 2 CH_2CH_2), 3.08 (m, 2H, $BocNHCH_2$), 3.40 (s, 2H, $ArCH_2$), 3.50 (s, 4H, 2 $ArCH_2$), 3.64 (s, 2H, $ArCH_2$), 5.55 (br s, 1H, BocNH), 7.28 (m, 20H, ArH).

Anal. Calcd. for $C_{42}H_{57}N_5O_2$: C, 75.98; H, 8.65; N, 10.55. Found: C, 75.72; H, 8.67; N, 10.39.

13-Amino-1,4,7,10-tetra(phenylmethyl)-1,4,7,10-tetraazatridecane (**2**).

To a stirred solution of **11** (2.65 g, 4 mmoles) in dichloromethane (10 ml) was added trifluoroacetic acid (10 ml) at room temperature. The reaction mixture was allowed to stir at room temperature for 30 minutes and evaporated to dryness. The residue was dissolved in dichloromethane (100 ml) and washed with 5% sodium bicarbonate solution (150 ml) to *p*H 8, and brine (50 ml). The organic extract was dried over anhydrous sodium sulfate and concentrated to dryness. The oily residue that obtained was used as such for the next reaction; ^{1}H nmr (deuteriochloroform): δ 1.50 (m, 5H, $CH_2CH_2CH_2$, NH_2, and $ArCH_2NH$), 2.38 (t, 2H, J = 6.4 Hz, CH_2NHCH_2), 2.54 (m, 14H, 7 CH_2), 3.52 (s, 2H, $ArCH_2$), 3.56 (s, 4H, 2 $ArCH_2$), 3.62 (s, 2H, $ArCH_2$), 7.28 (m, 20H, ArH).

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-*N*-[4,7,10,13-tetrakis(phenylmethyl)-4,7,10,13-tetraazatridec-1-yl]-2′-deoxyguanosine (**3**).

A mixture of 2-chloroinosine (**1**, 2.12 g, 4 mmoles) and the amine **2** (2.5 g, 4.4 mmoles) in 2-methoxyethanol (50 ml) was heated at 80° for 12 hours. The reaction mixture was evaporated to dryness and the residue on flash chromatography over silica gel using dichloromethane and methanol (9:1) gave 2.55 g (60%) of the titled compound as foam; ^{1}H nmr (deuteriochloroform): δ 1.00 (m, 24H, 4 isobutyl-H), 1.62 (m, 1H, $C_{2'}H$), 1.80 (m, 4H, $CH_2CH_2CH_2$, $C_{2'}H$, and $ArCH_2NH$), 2.52 (m, 14H, 7 CH_2), 3.20 (s, 2H, $ArCH_2$), 3.32 (s, 2H, $ArCH_2$), 3.42 (s, 2H, $ArCH_2$), 3.48 (s, 4H, $ArCH_2$, and CH_2), 3.78 (m, 1H, $C_{4'}H$), 4.05 (m, 2H, $C_{5'}CH_2$), 4.72 (m, 1H, $C_{3'}H$), 6.22 (m, 1H, $C_{1'}H$), 6.94 (m, 1H, N_2H), 7.26 (m, 20H, ArH), 7.72 (s, 1H, C_8H), 10.52 (br s, 1H, NH).

Anal. Calcd. for $C_{59}H_{85}N_9O_5Si_2$: C, 67.07; H, 8.11; N, 11.93. Found: C, 67.22; H, 8.24; N, 11.81.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-6-*O*-(phenylmethyl)-*N*-[15-methyl-14-oxo-4,7,10,13-tetrakis(phenylmethyl)-4,7,10,13-tetraazahexadec-1-yl]-2′-deoxyguanosine (**12**).

Compound **3** (2.00 g, 1.89 mmoles) was coevaporated with dry pyridine (30 ml) twice. The resulting residue was dissolved in dry pyridine (50 ml) and cooled to 0° in an ice bath mixture. To this cold stirred solution was added triethylamine (0.61 g, 6 mmoles) followed by isobutyryl chloride (0.64 g, 6 mmoles) slowly under an argon atmosphere. After the addition of isobutyryl chloride, the reaction mixture was stirred at room temperature for 12 hours and evaporated to dryness. The residue was dissolved in dichloromethane (150 ml), washed with 5% sodium bicarbonate (50 ml), water (50 ml) and brine (50 ml). The organic extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue on purification over silica gel using dichloromethane/methanol (95:5) gave 1.88 g (88%) of the isobutyl derivative of **3** as foam.

The above foam (1.8 g, 1.61 mmoles) was dried over phosphorus pentoxide under vacuum for 12 hours. The dried residue was dissolved in dry dioxane (50 ml) and treated with triphenyl phosphine (0.83 g, 3.2 mmoles), benzyl alcohol (0.35 g, 3.2 mmoles), and diethyl azodicarboxylate (0.54 g, 3.2 mmoles) at room temperature under argon atmosphere. The reaction mixture after stirring for 10 hours evaporated to dryness. The residue was dissolved in dichloromethane (150 ml) and washed with 5% sodium bicarbonate (50 ml), water (50 ml) and brine (50 ml). The organic extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was flash chromatographed over silica gel using dichloromethane/acetone (7:3) as the eluent. The pure fractions were collected together and evaporated to give 1.7 g (74%) of **12** as foam: ^{1}H nmr (deuteriochloroform): δ

1.04 (m, 30H, 5 isobutyl-CH_3), 1.68 (m, 2H, $CH_2CH_2CH_2$), 2.55 (m, 16H, 7 CH_2, $C_{2'}H$, and isobutyl-CH), 3.08 (m, 1H, $C_{2'}H$), 3.36 (m, 2H, CH_2), 3.52 (m, 8H, 4 ArCH_2), 3.84 (m, 1H, $C_{4'}H$), 4.00 (m, 2H, $C_{5'}H_2$), 4.72 (m, 1H, $C_{3'}H$), 5.50 (s, 2H, ArCH_2), 6.18 (m, 1H, $C_{1'}H$), 7.04 (m, 1H, N_2H), 7.26 (m, 25H, ArH), 7.76 (s, 1H, C_8H).

Anal. Calcd. for $C_{70}H_{97}N_9O_6Si_2$: C, 69.09; H, 8.04; N, 10.36. Found: C, 69.12; H, 8.23; N, 10.19.

6-*O*-(Phenylmethyl)-*N*-[15-methyl-14-oxo-4,7,10,13-tetrakis(phenylmethyl)-4,7,10,13-tetraazahexadec-1-yl]-2′-deoxyguanosine (**4a**).

To a stirred solution of the substrate **12** (5.0 g, 4.11 mmoles) in pyridine (50 ml) was added freshly prepared 1*N* solution of tetrabutylammonium fluoride (20 ml, 20 mmoles, prepared in a mixture of pyridine:tetrahydrofuran:water in the ratio of 5:4:1) at room temperature. The reaction mixture was allowed to stir for 30 minutes and quenched with H^+ resin (pyridinium form) to *p*H 6-7. The resin was filtered, washed with methanol (50 ml) and the combined filtrate evaporated to dryness. The residue was dissolved in dichloromethane (200 ml), washed with water (50 ml), and brine (50 ml). The organic extract was dried over sodium sulfate and concentrated to dryness. The foam obtained was purified by flash chromatography over silica gel column using dichloromethane/methanol (95:5) as the eluent. The required fractions were collected together and evaporated to give 3.5 g (87%) of the title compound as a foam; ^{1}H nmr (deuteriochloroform): δ 1.04 (m, 30H, 5 isobutyryl CH_3), 1.68 (m, 2H, $CH_2CH_2CH_2$), 2.55 (m, 16H, 7 CH_2, $C_{2'}H$, and isobutyryl CH), 3.08 (m, 1H, $C_{2'}H$), 3.36 (m, 2H, CH_2), 3.52 (m, 8H, 4 ArCH_2), 3.84 (m, 1H, $C_{4'}H$), 4.00 (m, 2H, $C_{5'}CH_2$), 4.72 (m, 1H, $C_{3'}H$), 5.50 (s, 2H, ArCH_2), 6.18 (m, 1H, $C_{1'}H$), 7.04 (m, 1H, N_2H), 7.26 (m, 25H, ArH), 7.76 (s, 1H, C_8H).

Anal. Calcd. for $C_{70}H_{97}N_9O_6Si_8$: C, 69.09; H, 8.04; N, 10.36. Found: C, 69.12; H, 8.23; N, 10.19.

REFERENCES AND NOTES

[1] D. M. Huryn and M. Okabe, *Chem. Rev.*, **92**, 1745 (1992).

[2] R. K. Robins, *Chem. Eng. News*, **64**, 28 (1986).

[3] P. D. Cook, Medicinal Chemistry Strategies for Antisense Research in Antisense Research and Applications, S. T. Crooke, B. LeBleu eds, CRC Press, Inc., Boca Raton, FL, 1993.

[4] K. Niiya, R. A. Olsson, R. D. Thompson, S. K. Silvia and M. Ueeda, *J. Med. Chem.*, **35**, 4557 (1992).

[5] S. Freese, J. Hoheisel, H. Lehrach and G. Wright, *Nucleosides Nucleotides*, **10**, 1507 (1991).

[6] J. Gambino, F. Focher, C. Hildebrand, G. Maga, T. Noonan, S. Spadari and G. Wright, *J. Med. Chem.*, **35**, 2979 (1992).

[7] R. Casale and L. W. McLaughlin, *J. Am. Chem. Soc.*, **112**, 5264 (1990).

[8] K. Ramasamy, M. Zounes, C. Gonzalez, S. M. Freier, E. A. Lesnik, L. L. Cummins, R. H. Griffey, B. P. Monia and P. D. Cook, *J. Am. Chem. Soc.*, submitted (1993).

[9] J.-P. Lenoetti, G. Degols and B. Lebleu, *Bioconjugate Chem.*, **1**, 149 (1990).

[10] M. Crampolini and P. Paoletti, *J. Phys. Chem.*, **65**, 1224 (1961).

[11] A. Vacca, D. Arenare and P. Paoletti, *Inorg. Chem.*, **5**, 1384 (1966).

[12] K. L. Garraway, P. C. Huang and T. G. Scott, Synthetic Procedures in Nucleic Acid Chemistry, Vol **1**, W. W. Zorbach and R. S. Tipson, eds, John Wiley and Sons, New York, **1**, 1968, p 3.

[13] R. J. Bergeron, J. R. Garlich and H. J. Stolowich, *J. Org. Chem.*, **49**, 2997 (1984).

[14] T. Ando and J. Yamawaki, *Chem. Letters*, 45 (1979).

[15] J. S. Bradshaw, K. E. Krakowiak and R. M. Izatt, *Tetrahedron*, **48**, 4475 (1992).

[16] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala and W. Pfleiderer, *Tetrahedron*, **40**, 59 (1984).