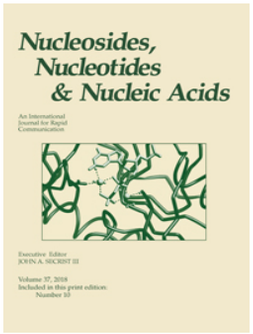


Nucleosides, Nucleotides and Nucleic Acids

ISSN: 1525-7770 (Print) 1532-2335 (Online) Journal homepage: https://www.tandfonline.com/loi/lncn20

A convenient route to synthesize N^2-(isobutyryl)-9-(carboxymethyl)guanine for *aeg*-PNA backbone

Dhrubajyoti Datta

To cite this article: Dhrubajyoti Datta (2019): A convenient route to synthesize N^2-(isobutyryl)-9-(carboxymethyl)guanine for *aeg*-PNA backbone, Nucleosides, Nucleotides and Nucleic Acids, DOI: 10.1080/15257770.2019.1666139

To link to this article: https://doi.org/10.1080/15257770.2019.1666139

Published online: 07 Oct 2019.

NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACIDS
https://doi.org/10.1080/15257770.2019.1666139

A convenient route to synthesize N^2-(isobutyryl)-9-(carboxymethyl)guanine for *aeg*-PNA backbone

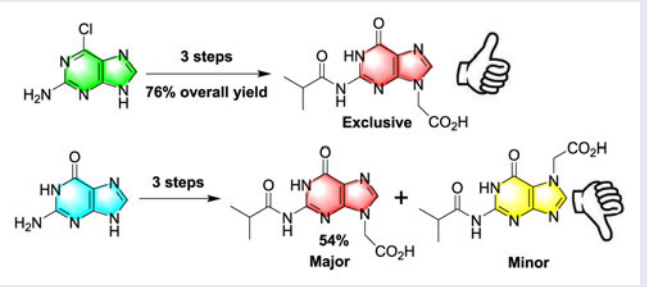
Dhrubajyoti Datta

Department of Chemistry, Chemical Biology Unit, Indian Institute of Science Education and Research, Pune, India

ABSTRACT

Synthesis of exclusive N^2-(isobutyryl)-9-(carboxymethyl)guanine, an important moiety for peptide nucleic acid synthesis has been reported through a high-yielding reaction scheme starting from 6-chloro-2-amino purine. Crystal structures of two intermediates confirmed the formation of *N*9-regioisomer. This new synthetic route can potentially replace the conventional tedious method with moderate overall yield.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 22 November 2018
Accepted 6 September 2019

KEYWORDS
Peptide nucleic acid; guanine; N9-regioisomer; crystal structure

1. Introduction

Organic biopolymer like nucleic acid is well recognized for chemical/molecular evolution of life. Self-recognition and self-assembly of nucleic acids are mainly driven by complementary (adenine:thymine; A:T and guanine:cytosine; G:C) and homo base pairings ($C:C^+$ and G:G). DNA and RNA are well known for self-assembly in nanometric dimension and have unfurled one of the most rapidly expanding areas of research, with a wide range of applications from therapeutics to drug delivery and sensors.[1] Peptide nucleic acid (PNA) is a DNA mimic in which the sugar–phosphate backbone is replaced by aminoethyl–glycyl (*aeg*) backbone to which the

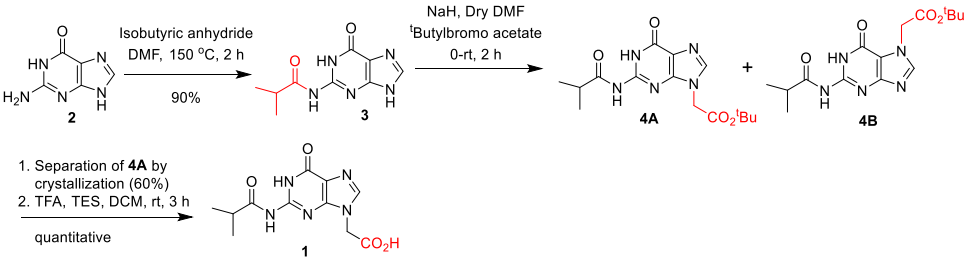
CONTACT Dhrubajyoti Datta dhrubajyoti@iiserpune.ac.in Department of Chemistry, Chemical Biology Unit, Indian Institute of Science Education and Research, Pune, 411008 India.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lncn.
Supplementary material for this article can be accessed at https://doi.org/10.1080/15257770.2019.1666139.

nucleobases A/G/C/T are connected by acetyl spacer through a tertiary amide linkage.[2] PNA binds to complementary DNA/RNA (cDNA/cRNA) better than the corresponding DNA with its cDNA/cRNA and with high sequence specificity.[3] Simultaneously, interesting structural motifs derive from homomeric combinatorial G-duplex, tetraplexes (G-tetrad/G-quadruplex) and i-motifs ($C:C^+$) owing to various non-canonical structures.[4]

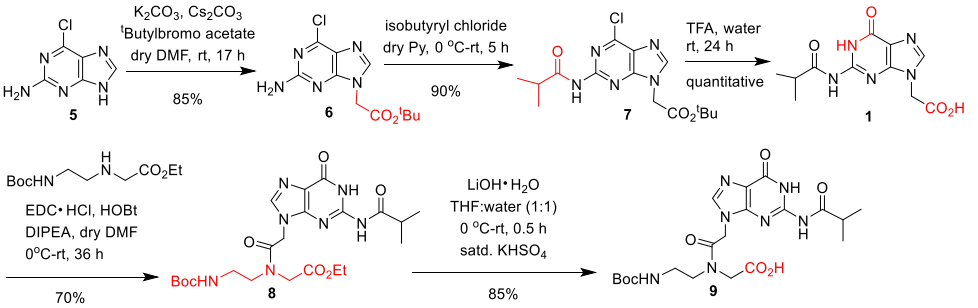
The marked effect regioisomer on PNA backbone was demonstrated by D'Costa et al.[5] when *N*7-G was incorporated as a protonated cytosine mimic. Hence, unlike cytosine or pseudo-isocytosine, *N*7-guanine cannot interact through Watson–Crick (W–C) hydrogen bonding. Later, duplex/triplex formation through a (*S,S*)-*trans*-cyclopentane (*tcyp*) modified PNA backbone was investigated by Englund et. al.[6] using both *N*7 and *N*9-guanine isomers. For *aeg*–G–PNA synthesis, the preparation of the monomers requires a suitably protected *aeg*, and the guanine acetic acid derivatives. Among all the nucleobase acetic acids, preparation of the guanine acetic acid derivatives pose the greatest synthetic challenge.[7] So far, the only route which yielded exclusive *N*9-regioisomer of G-acetic acid was reported by Uhlmann et al.[8] Unfortunately, it requires *N2*-acetyl-*O4*-dipheylcaxbamoylguanine,[9] a derivative of guanine which is difficult to scale up quickly. Nevertheless, the regiomeric purity of G-PNA cannot be compromised as *N*9-regioisomer is faceted to accommodate complementary nucleobase C through either W–C or Hoogsteen site. It was reported that synthesis of N^2-(isobutyryl)-9-(carboxymethyl)guanine **1** from guanine is difficult due to 'time-consuming separation process' of *N*9- and *N*7-regioisomers.[10] However, lowering the temperature favored the formation of the desired *N*9-regioisomer.[8,11] The separation of *N*9/*N*7 regioisomers through chromatographic technique is extremely laborious due to small R_f difference between the regioisomers. To overcome these drawbacks, the author has proposed a modified route which starts from a relatively expensive purine nucleobase 6-chloro-2-aminopurine which in turn can diminish the selectivity problem[7] in alkylation reactions. Earlier, *N*9 regioisomer of guanine derivatives were successfully synthesized by Harnden et al.[12] employing 6-chloro analogues which prompted us to revisit and attend this problem.

2. Results and discussion

Initially, **1** was synthesized following the literature procedure[6,10,13] to afford a moderate overall yield of 54% with respect to guanine **2**. N^2-Isobutyrylguanine **3**[14] was synthesized first from **2** which was alkylated using NaH/*tert*-butylbromoacetate at low temperature to afford *N*9-alkylated derivative **4A** with *N*7 regioisomer **4B**[11] (Scheme 1). Desired



Scheme 1. Conventional synthesis of N^2-(isobutyryl)-9-(carboxymethyl)guanine **1**.



Scheme 2. Modified synthetic route of **1** and its consequent attachment on *aeg*–PNA backbone.

isomer **4A** was obtained after a flash column chromatography followed by recrystallization of crude regiomeric mixture. Accumulation of two crops of white crystals from ethyl acetate (EtOAc) within 3 days afforded 60% pure **4A**. Removal of the *tert*-butyl ester group of **4A** using trifluoroacetic acid (TFA) in presence of triethylsilane (TES) produced the *N*9-guanine acetic acid **1** in quantitative yield.

In modified route, alkylation of 6-chloro-2-aminopurine **5** by *tert*-butyl-bromoacetate under mild basic conditions afforded *N*9 isomer **6**[15] as exclusive product with high yield at room temperature. None of the previous literatures[15] did report the *N*9 isomer of **6** through single crystal structure. However, structure of *tert*-butyl[2-(((benzyloxy)carbonyl)(*tert*-butoxycarbonyl)amino)-6-(2-nitrophenoxy)-9*H*-purin-9-yl]acetate,[16] a derivative of **6**, was reported. The author proceeded further to protect the 2-amino group of **6** with isobutyryl chloride to afford **7** in good yield. Treatment of **7** with 50% aqueous TFA[17] yielded desired N^2-(isobutyryl)-9-(carboxymethyl)guanine **1** with 76% overall yield with respect to **5** (Scheme 2). Guanine acetic acid thus obtained was coupled with *aeg*-PNA backbone in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxybenzotriazole (HOBt) to afford Boc-*aeg*-(G^{ibu})-PNA-OEt (**8**) in good yield. Ester **8**, thus obtained, was hydrolyzed under basic condition to afford the G-PNA monomer Boc-*aeg*-(G^{ibu})–PNA–OH (**9**) monomer in good yield which was used to prepare a representative PNA (**pG_5**) on solid phase using Boc-strategy (SI).[18]

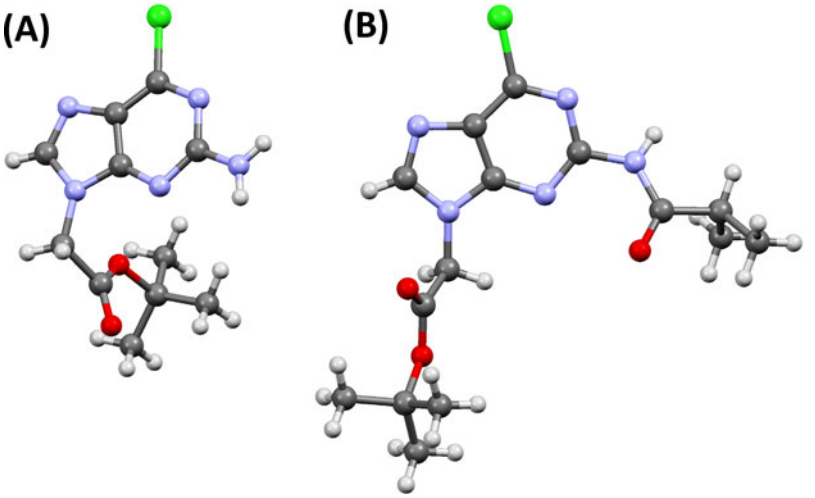


Figure 1. Crystal structure of (A) *tert*-butyl(2-amino-6-chloropurin-9-yl)acetate **6** and (B) *tert*-butyl[2-(isobutyryl)amino-6-chloropurin-9-yl]acetate **7** showing *N*9 isomer.

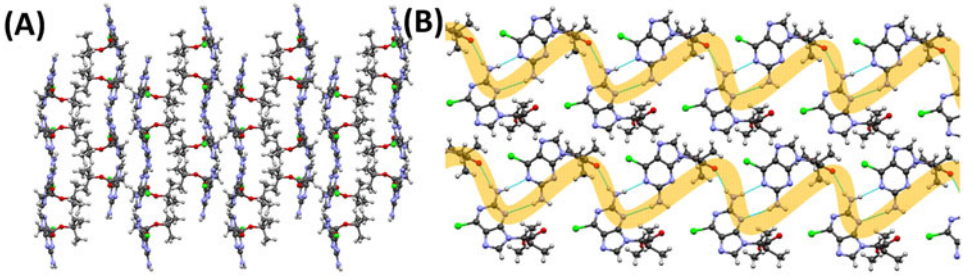


Figure 2. (A) Packing (2 × 2×2) along *a*-axis and (B) H-bond mediated ribbon like two-dimentional array observed in single crystal structure along *b*-axis for intermediate **6**.

Intermediate **6** was crystallized from a 1:1 mixture of EtOAc and petroleum ether as yellowish white needles while excellent rod like crystals of **7** was seen from chloroform. Single crystal structures of intermediates **6** (CCDC 1880599) and **7** (CCDC 1880600) confirmed the formation of desired isomer **1** (Figure 1). It was noteworthy that both **6** and **7** were purified thorough simple column chromatography through silica gel and the solid pure compounds thus obtained, were crystallized easily in aforementioned solvent systems. Different modes of H-bondings were observed inside these crystals (Figures 2 and 3). For compound **6**, eight-member ring formation was seen between *N*1, *N*3 and NH_2 of two adjacent guanine bases (NH_2··· *N*1 distance: 2.44 Å and NH_2··· *N*3 distance: 2.97 Å). Apart from that another H-bonding interaction was found observed between the C=O of *tert*-butyl ester and NH_2 groups (C=O ···NH_2 distance: 2.38 Å) (Figure 2B). On the other hand, intermolecular H-bonding was found between *iso*butyryl group protected amide N*H* and *N*7 of two adjacent

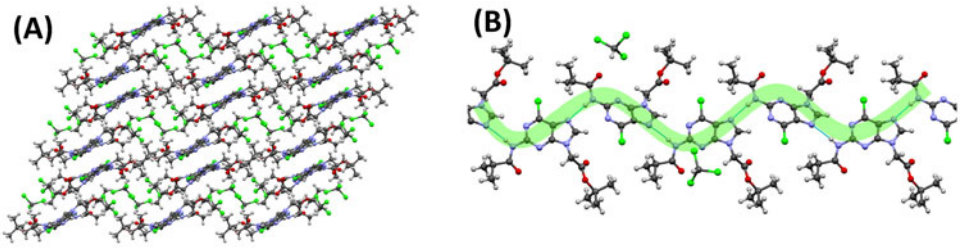


Figure 3. (A) Packing (3 × 3×3) along *b*-axis incorporating $CHCl_3$ molecules and (B) wavy intermolecular H-bond observed in single crystal structure along *a*-axis for intermediate **7**.

guanines of **7** (average distance between amide N*H* ···*N*7 distance: 2.25 Å) (Figure 3B). ^{1}H NMR data of **1** obtained from Schemes 1 and 2 exhibit identical chemical shift values. Characteristic proton attached to C8 of guanine appeared at 8.0 ppm whereas N*H* protons of *N*1 and 2-amino group were found at 12.1 and 11.7 ppm, respectively (Figure 4). NMR datum for **1** recorded in DMSO-d_6 was in complete agreement with the reported value of *N*9 regioisomer.

3. Conclusions

In conclusion, the author has successfully synthesized N^2-(isobutyryl)-9-(carboxymethyl)guanine **1** exclusively through a modified synthetic route which afforded regioselectively *N*9-analog with higher yields compared to that of conventional procedure. Moreover, it reduces the reaction time appreciably to make this important building block. N^2-(Isobutyryl)-9-(carboxymethyl)guanine obtained from this modified scheme was attached to the *aeg* PNA backbone to obtain the G-PNA monomer Boc-*aeg*-(G^{ibu})–PNA–OH which has successfully been used for Boc[18]-strategy in solid phase peptide synthesis (SPPS). This new reaction route is expected to facilitate synthesis of PNA other organic molecules containing guanine, the toughest among all other nucleobases.

4. Experimental section

4.1. General Experimental Procedure

All reagents were commercially purchased from Sigma Aldrich. Column chromatographic separations were done using silica gel (100–200 mesh). Solvents were dried and distilled following standard procedures. TLC was carried out on pre-coated plates (Merck silica gel 60, f_{254}) and the spots were visualized with UV light. ^{1}H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on Bruker and Jeol NMR spectrometer. All ^{1}H and 13C NMR were recorded in $CDCl_3$ and DMSO-d_6. Chemical

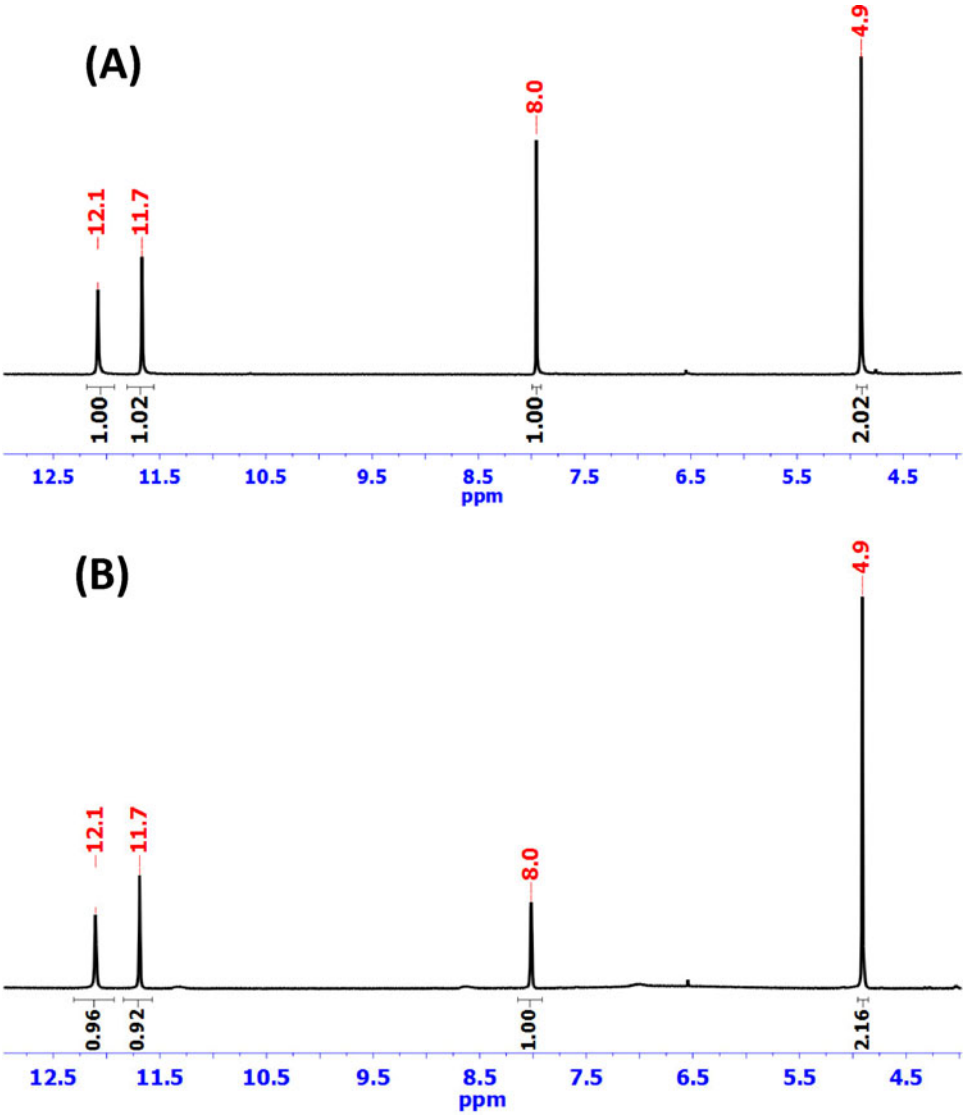


Figure 4. ^{1}H NMR of **1** afforded from (A) conventional and (B) modified synthetic route in DMSO-d_6 recorded in 400 MHz instrument showing characteristic signals for *N*9-regioisomer.

shifts are reported in parts per million (ppm, δ scale). If tetramethylsilane (TMS) was not present in the deuterated solvent, the residual protonated solvent is referenced ($CDCl_3$, δ 7.26; DMSO-d_6, δ 2.50). Mass spectroscopy data were obtained from Synapt G2QTof mass spectrometer in ESI^+ mode. Melting points were determined in open-end capillary tubes and are uncorrected. Single crystal X-ray diffraction (SCXRD, Bruker), UV–Visible spectrophotometry (Perkin Elmer Lambda 45 double beam UV–Vis spectrophotometer) data were collected for compound characterization.

4.2. SCXRD study

4.2.1. General procedure for crystallization

Single crystals X-ray studies were performed in Bruker D8 Venture single crystal diffractometer in 4-circle goniometer configuration, equipped with dual wavelength (Cu and Mo). InCoatec microfocus X-ray sources and Photon 100 detector was used for data collection. Oxford Cryosystems' Cryostream plus low temperature attachment (80–500 K) was used to keep crystal at 100 K during data collection.

Samples were purified carefully before keeping for crystallization. Glass sample vials (2 mL) were washed with acetone and dried under a nitrogen gas stream before use. PARAFILM "M" was used to close the vials. HPLC-grade solvents were used for crystallization. The investigated single crystal was a small-sized and the quality of diffraction was good. Numerous datasets were collected on single crystals from different batches and one of the highest qualities is reported herein. All non-hydrogen atoms were refined anisotropically. H-atoms were placed at calculated positions and refined as riding atoms with isotropic displacement parameters. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre and the deposition numbers are indicated.

4.3. Synthesis

4.3.1. tert-Butyl-(2-amino-6-chloropurin-9-yl)acetate 6

2-Amino-6-chloropurine **5** (1.02 g, 6.0 mmol), K_2CO_3 (0.87 g, 6.3 mmol) and Cs_2CO_3 (0.21 g, 0.63 mmol) were stirred under N_2, in DMF (20 mL). Then *tert*-butyl bromoacetate (0.93 mL, 6.3 mmol) was added dropwise, and the resulting mixture was stirred for 17 h. The reaction mixture was suspended in water (100 mL) and the aq. layer was extracted with EtOAc (50 × 3 mL). The combined organic layer was washed with water and brine solution. The organic layer was separated, dried over anhyd Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude mass thus obtained was purified by column chromatography [Eluent: 20–70% of EtOAc in petroleum ether] to afford compound **6** (1.45 g, 85%). Yellowish white solid; m.p. 163–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) $\delta = 8.09$ (s, 1 H), 6.96 (s, 2 H), 4.85 (s, 2 H), 1.41 ppm (s, 9 H); 13C NMR (100 MHz, DMSO-d_6, 25 °C) $\delta = 166.7$, 159.9, 154.3, 149.4, 143.6, 122.9, 82.3, 44.6, 27.7 ppm. HRMS (ESI$^+$), *m*/*z* calculated for (M + H) $C_{11}H_{15}ClN_5O_2$: 284.0914, found: 284.0916.

4.3.2. tert-Butyl[2-(isobutyryl)amino-6-chloropurin-9-yl]acetate 7

To a solution of *tert*-butyl-(2-amino-6-chloropurin-9-yl)acetate **6** (1.0 g, 3.53 mmol) in pyridine (20 mL) at 0 °C was added isobutyrylchloride (0.4 g,

3.75 mmol) at 0 °C. The mixture was kept at 0 °C for 1 h and then followed by stirring at room temperature for 5 h. Saturated $NaHCO_3$ solution (50 mL) was added to the reaction mixture followed by EtOAc (50 mL) and the organic layer was partitioned. Aqueous part was washed with EtOAc (2 × 25 mL). The combined organic layer was dried over anhyd. Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude mass thus obtained was purified by column chromatography [Eluent: 10–50% EtOAc in petroleum ether] to afford compound **7** (1.12 g, 90%). Yellowish white solid; m.p. 166–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) $\delta = 8.12$ (bs, 1 H), 8.09 (s, 1 H), 4.90 (s, 2 H), 3.04–2.87 (m, 1 H), 1.49 (s, 9 H), 1.28 ppm (d, $J = 6.9$ Hz, 6 H); 13C NMR (100 MHz, $CDCl_3$, 25 °C, TMS) $\delta = 175.8$, 165.7, 153.1, 152.2, 151.3, 145.2, 127.7, 84.2, 45.2, 36.1, 28.1, 19.4 ppm; HRMS (ESI^+), *m/z* calculated for (M + H) $C_{15}H_{21}ClN_5O_3$: 354.1333, found: 354.1338.

4.3.3. N^2-(Isobutyryl)-9-(carboxymethyl)guanine 1

Compound **7** (1.0 g) was dissolved in 3:1 mixture of TFA:H_2O (10 mL) and then stirred for 24 h at room temperature. The reaction mixture was evaporated and triturated well with chilled diethyl ether to afford N^2-(isobutyryl)-9-(carboxymethyl)guanine **1** (0.79 g, quantitative yield). White solid; m.p. 200–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) $\delta = 12.10$ (s, 1 H), 11.69 (s, 1 H), 8.02 (s, 1 H), 4.91 (s, 2 H), 2.82–2.71 (m, 1 H), 1.11 ppm (d, $J = 6.8$ Hz, 6 H); 13C NMR (100 MHz, DMSO-d_6, 25 °C) $\delta = 180.2$, 169.0, 154.7, 148.9, 148.2, 140.3, 119.3, 44.5, 34.7, 18.9 ppm. HRMS (ESI^+), *m/z* calculated for (M + H) $C_{11}H_{14}N_5O_4$: 280.1046, found: 280.1052.

4.3.4. Boc–aeg–(G^{ibu})–PNA–OEt 8

Compound **8** was synthesized following the known literature procedure[6] as white solid. M.p. 110–115 °C; ^{1}H NMR, (400 MHz, DMSO-d_6, 25 °C) $\delta = 12.08$ (d, $J = 5.5$ Hz, 1 H), 11.65 (d, $J = 7.6$ Hz, 1 H), 7.83 (s, 0.7 H), 7.82 (s, 0.3 H), 7.03 (t, $J = 5.5$ Hz, 0.7 H), 6.76 (s, 0.3 H), 5.13 (s, 1.4 H), 4.97 (s, 0.6 H), 4.42 (s, 0.6 H), 4.22 (q, $J = 7.1$ Hz, 0.6 H), 4.08 (q, $J = 7.0$ Hz, 2.8 H), 3.50 (t, $J = 6.5$ Hz, 1.4 H), 3.32 (d, $J = 11.9$ Hz, 0.6 H), 3.24 (d, $J = 6.1$ Hz, 1.4 H), 3.02 (d, $J = 6.1$ Hz, 0.6 H), 2.84–2.71 (m, 1 H), 1.37 (d, $J = 6.5$ Hz, 9 H), 1.27 (t, $J = 7.1$ Hz, 1 H), 1.17 (t, $J = 7.1$ Hz, 2 H), 1.11 ppm (d, $J = 6.7$ Hz, 6 H); 13C NMR (100 MHz, DMSO-d_6, 25 °C) $\delta = 180.2$, 180.1, 169.5, 169.0, 167.0, 166.6, 155.8, 155.6, 154.9, 149.1, 149.2, 147.9, 147.9, 140.5, 140.4, 119.6, 78.2, 77.8, 61.3, 60.6, 49.1, 47.8, 46.9, 44.1, 43.9, 38.3, 37.7, 34.7, 34.7, 28.2, 18.9, 14.0 ppm. HRMS (ESI^+), m/z calculated for (M + H) $C_{22}H_{34}N_7O_7$: 508.2520, found: 508.2535.

4.3.5. Boc-aeg-(G^{ibu})–PNA–OH 9

To a solution of compound **8** (0.25 g, 0.49 mmol) in tetrahydrofuran (10 mL) and distilled water (10 mL) was added $LiOH{\cdot}H_2O$ (0.03 g, 0.74 mmol) at 0 °C and the reaction mixture was stirred for 0.5 h. The aq layer was adjusted to pH 3–4 with satd $KHSO_4$ at 0 °C and then extracted with EtOAc (4 × 35 mL). The combined organic layer was dried over anhyd Na_2SO_4 and filtered. The filtrate was evaporated *in vacuo* and crude product was purified by column chromatography [Eluent: 5–15% MeOH in chloroform ($CHCl_3$)] to afford compound **9** (0.20 g, 85%) as a white solid. M.p. 205–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) $\delta = 12.14$ (s, 0.3 H), 12.12 (s, 0.7 H), 11.75 (s, 0.3 H), 11.70 (s, 0.7 H), 7.97 (s, 0.3 H), 7.95 (s, 0.7 H), 7.05 (t, $J = 5.5$ Hz, 0.7 H), 6.78 (t, $J = 5.2$ Hz, 0.3 H), 5.14 (s, 1.4 H), 4.98 (s, 0.6 H), 4.32 (s, 0.6 H), 4.00 (s, 1.4 H), 3.48 (t, $J = 6.6$ Hz, 1.4 H), 3.32 (t, $J = 6.8$ Hz, 0.6 H), 3.24 (dd, $J = 13.2$, 7.2 Hz, 1.4 H), 3.07–2.98 (m, 0.6 H), 2.83–2.69 (m, 1 H), 1.36 (d, $J = 5.5$ Hz, 9 H), 1.11 (d, $J = 1.0$ Hz, 3 H), 1.10 ppm (s, 3 H); 13C NMR (100 MHz, DMSO-d_6, 25 °C) $\delta = 180.2$, 180.17, 170.8, 170.4, 166.8, 166.3, 155.8, 155.6, 154.6, 154.6, 149.2, 148.1, 140.6, 140.5, 118.94, 78.2, 77.8, 49.2, 48.6, 47.7, 47.0, 46.8, 44.3, 44.1, 38.2, 37.6, 34.7, 34.69, 28.2, 28.17, 18.7 ppm. HRMS (ESI^+), m/z calculated for (M + H) $C_{20}H_{30}N_7O_7$: 480.2206, found: 480.2213.

4.3.6. Note

Synthesis of compound **6** is the key reaction step in this work as it produced exclusive *N*9-regioisomer: *tert*-butyl-(2-amino-6-chloropurin-9-yl)acetate. The principal driving force behind exclusive *N*9-regioisomer formation is the stereo-electronic repulsion between the approaching bulky electrophile (*tert*-butylbromoacetate) and the Cl-atom at 6-position of 2-amino-6-chloropurine. *N*9-Nitrogen, in contrast is more accessible to the reagents under the reaction condition. Earlier, Debaene and Winssinger reported the synthesis of N^2-benzhydryloxycarbonyl (Bhoc) protected analog of **1** starting form 2-amino-6-chloropurine which involve a lengthy process[19] and suitable for only 9-fluorenylmethoxycarbonyl (Fmoc)-strategy[20] of SPPS. Although, K_2CO_3 alone was used to generated *N*9-regioisomer from 2-amino-6-chloropurine and 6-chloropurine,[19,21] in this report a mixture of K_2CO_3 and Cs_2CO_3 in 9:1 ratio to enhance the yield of alkylation reaction significantly. It is well known that Cs_2CO_3 is more soluble in polar organic solvents (*viz*., DMF, DMSO, NMP) than any other alkali-metal carbonates.[22] Simultaneously, the "cesium ion effect"[23] played important role to facilitate the *N*-alkylation of purine nucleobase. The ratio two bases (K_2CO_3 and Cs_2CO_3) was optimized after several trial reactions where an effective inorganic base (Cs_2CO_3) in organic reactions[24] improved the reaction yield at room temperature.

Acknowledgments

DD thanks Department of Chemistry, IISER Pune for instrumental facilities. DD is also thankful to Prof. Krishna N. Ganesh and DST SERB, India for National Post Doctoral Fellowship (Project Code: PDF/2016/002510).

Conflicts of interest

There are no conflicts of interest to declare.

ORCID

Dhrubajyoti Datta http://orcid.org/0000-0003-2077-1294

References

[1] (a) Meng, H.-M.; Liu, H.; Kuai, H.; Peng, R.; Mo, L.; Zhang, X.-B. Aptamer-integrated DNA nanostructures for biosensing, bioimaging and cancer therapy. *Chem. Soc. Rev.* **2016**, *45*, 2583–2602. (b) Keyser, U. F. Enhancing nanopore sensing with DNA nanotechnology. *Nature Nanotech.* **2016**, *11*, 106–108.

[2] (a) Nielsen, P. E.; Haaima, G. Peptide nucleic acid (PNA). A DNA mimic with a pseudopeptide backbone. *Chem. Soc. Rev.* **1997**, *26*, 73–78. (b) Nielsen, P. E. Peptide Nucleic Acid. A Molecule with Two Identities. *Acc. Chem. Res.* **1999**, *32*, 624–630.

[3] Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. PNA Hybridizes to Complementary Oligonucleotides Obeying the Watson–Crick Hydrogen-Bonding Rules. *Nature* **1993**, *365*, 566–568. DOI: 10.1038/365566a0.

[4] (a) Egholm, M.; Christensen, L.; Dueholm, K. L.; Buchardt, O.; Coull, J.; Nielsen, P. E. *Nucleic Acids Res.* **1995**, 23, 217–222. (b) Datta, B.; Schmitt, C.; Armitage, B. A. *J. Am. Chem. Soc.* **2003**, 125, 4111–4118. (c) Datta, B.; Bier, M. E.; Roy, S.; Armitage, B. A. *J. Am. Chem. Soc.* **2005**, 127, 4199–4207. (d) Marin, V. L.; Armitage, B. A. *J. Am. Chem. Soc.* **2005**, *127*, 8032–8033.

[5] D'Costa, M.; Kumar, V. A.; Ganesh, K. N. N7-Guanine as a C + Mimic in Hairpin Aeg/aepPNA–DNA Triplex: Probing Binding Selectivity by UV-Tm and Kinetics by Fluorescence-Based Strand-Invasion Assay. *J. Org. Chem.* **2003**, *68*, 4439–4445. DOI: 10.1021/jo034048h.

[6] Englund, E. A.; Xu, Q.; Witschi, M. A.; Appella, D. H. PNA-DNA Duplexes, Triplexes, and Quadruplexes Are Stabilized with Trans-Cyclopentane Units. *J. Am. Chem. Soc.* **2006**, *128*, 16456–16457. DOI: 10.1021/ja064317w.

[7] Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. PNA: Synthetic Polyamide Nucleic Acids with Unusual Binding Properties. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2796–2823. DOI: 10.1002/(SICI)1521-3773(19981102)37:20<2796::AID-ANIE2796>3.0.CO;2-K.

[8] Breipohl, G.; Will, D. W.; Peyman, A.; Uhlmann, E. Novel Synthetic Routes to PNA Monomers and PNA–DNA Linker Molecules. *Tetrahedron* **1997**, *53*, 14671–14686. DOI: 10.1016/S0040-4020(97)01044-2.

[9] Zou, R.; Robins, M. High-Yield Regioselective Synthesis of 9-Glycosyl Guanine Nucleosides and Analogues via Coupling with 2-N-Acetyl-6-O-Diphenylcarbamoylguanine. *Can. J. Chem.* **1987**, *65*, 1436–1437. DOI: 10.1139/v87-243.

[10] Liu, Z.-C.; Shin, D.-S.; Lee, K.-T.; Jun, B.-H.; Kim, Y.-K.; Lee, Y.-S. Synthesis of Photolabile o-Nitroveratryloxycarbonyl (NVOC) Protected Peptide Nucleic Acid Monomers. *Tetrahedron* **2005**, *61*, 7967–7973. DOI: 10.1016/j.tet.2005.06.002.

[11] Timar, Z.; Kovacs, L.; Kovacs, G.; Schmel, Z. Fmoc / Acyl protecting groups in the synthesis of polyamide (peptide) nucleic acid monomers. *Perkin* **2000**, *1*, 19–26.

[12] Harnden, M. R.; Jarvest, R. L.; Bacon, T. H.; Boyd, M. R. Synthesis and Antiviral Activity of 9-[4-Hydroxy-3-(Hydroxymethyl)but-1-yl]Purines. *J. Med. Chem.* **1987**, *30*, 1636–1642. DOI: 10.1021/jm00392a020.

[13] Will, D. W.; Breipohl, G.; Langner, D.; Knolle, J.; Uhlmann, E. The Synthesis of Polyamide Nucleic Acids Using a Novel Monomethoxytrityl Protecting-Group Strategy. *Tetrahedron* **1995**, *51*, 12069–12082. DOI: 10.1016/0040-4020(95)00766-2.

[14] Jenny, T. F.; Schneider, K. C.; Benner, S. A. N2-Isobutyryl-O6-[2-(p-nitrophenyl)ethyl]guanine: a new building block for the efficient synthesis of carbocyclic guanosine analogs. *Nucleosides Nucleotides* **1992**, *11*, 1257–1261. DOI: 10.1080/07328319208018340.

[15] (a) Aldrian-Herrada, G.; Rabie, A.; Wintersteiger, R.; Brugidou, J. Solid-phase synthesis of peptide nucleic acid (PNA) monomers and their oligomerization using disulfide anchoring linkers. *J. Peptide Sci.* **1998**, 4, 266–281. (b) Heuer-Jungemann, A; Howarth, N. M.; JaAfaru, S. C.; Rosair, G. M. Development of a convenient route for the preparation of the N2-Cbz-protected guaninyl synthon required for Boc-mediated PNA synthesis. *Tetrahedron Lett.* **2013**, 54, 6275–6278. (c) Sato, N.; Tsuji, G.; Sasaki, Y.; Usami, A.; Moki, T.; Onizuka, K.; Yamada, K.; Nagatsugi, F. A new strategy for site-specific alkylation of DNA using oligonucleotides containing an abasic site and alkylating probes. *Chem. Commun.* **2015**, *51*, 14885–14888.

[16] Heuer-Jungemann, A.; Howarth, N. M.; Ja'Afaru, S. C.; Rosair, G. M. Development of a Convenient Route for the Preparation of the N2-Cbz-Protected Guaninyl Synthon Required for Boc-Mediated PNA Synthesis. *Tetrahedron Lett.* **2013**, *54*, 6275–6278. DOI: 10.1016/j.tetlet.2013.09.034.

[17] Nagapradeep, N.; Verma, S. Characterization of an Unprecedented Organomercury Adduct via Hg(II)-Mediated Cyclization of N9-Propargylguanine. *Chem. Commun. (Camb.)* **2011**, *47*, 1755–1757. DOI: 10.1039/c0cc03123b.

[18] (a) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. Peptide nucleic acids (PNA). Oligonucleotide analogs with an achiral peptide backbone. *J. Am. Chem. Soc.* **1992**, *114*, 1895–1897. (b) Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H. Recognition of guanine and adenine in DNA by cytosine and thymine containing peptide nucleic acids (PNA). *J. Am. Chem. Soc.* **1992**, *114*, 9677–9678. DOI: 10.1021/ja00050a068.

[19] Debaene, F.; Winssinger, N. Azidopeptide Nucleic Acid. An Alternative Strategy for Solid-Phase Peptide Nucleic Acid (PNA) Synthesis. *Org. Lett.* **2003**, *5*, 4445–4447. DOI: 10.1021/ol0358408.

[20] (a) Neuner, P.; Monaci, P. New Fmoc Pseudoisocytosine Monomer for the Synthesis of a Bis-PNA Molecule by Automated Solid-Phase Fmoc Chemistry. *Bioconjugate Chem.* **2002**, 13, 676–678. (b) Porcheddu, A.; Giacomelli, G.; Piredda, I.; Carta, M.; Nieddu, G. A practical and efficient approach to PNA monomers compatible with Fmoc-mediated solid-phase synthesis protocols. *Eur. J. Org. Chem.* **2008**, 5786–5797. (c) Avitabile, C.; Moggio, L.; D'Andrea, L. D.; Pedone, C.; Romanelli, A. Development of an efficient and low-cost protocol for the manual PNA synthesis by Fmoc chemistry. *Tetrahedron Lett.* **2010**, 51, 3716–3718. (d) Behrendt, R.; White, P.; Offer, J. Advances in Fmoc solid-phase peptide synthesis. *J. Pept. Sci.* **2016**, 22,

4–27. (e) Luna, O. F.; Gomez, J.; Cardenas, C.; Albericio, F.; Marshall, S. H.; Guzman, F. Deprotection reagents in Fmoc solid phase peptide synthesis: Moving away from piperidine? *Molecules* **2016**, 21, 1542. (f) Sugiyama, T.; Hasegawa, G.; Niikura, C.; Kuwata, K.; Imamura, Y.; Demizu, Y.; Kurihara, M.; Kittaka, A. PNA monomers fully compatible with standard Fmoc-based solid-phase synthesis of pseudocomplementary PNA. *Bioorg. Med. Chem. Lett*. **2017**, *27*, 3337–3341.

[21] Qu, G.; Zhang, Z.; Guo, H.; Geng, M.; Xia, R. Microwave-Promoted Facile and Efficient Preparation of N-(Alkoxycarbonylmethyl) Nucleobases-Building Blocks for Peptide Nucleic Acids. *Molecules* **2007**, *12*, 543–551. DOI: 10.3390/12030543.

[22] Cella, J. A.; Bacon, S. W. Preparation of Dialkyl Carbonates via the Phase-Transfer-Catalyzed Alkylation of Alkali Metal Carbonate and Bicarbonate Salts. *J. Org. Chem.* **1984**, *49*, 1122–1125. DOI: 10.1021/jo00180a033.

[23] (a) Dijkstra, G.; Kruizinga, W. H.; Kellogg, R. M. An assessment of the causes of the "cesium effect". *J. Org. Chem*. **1987**, 52, 4230–4234. (b) Galli, C. "Cesium ion effect" and macrocyclization. A critical review. *Org. Prep. Proced. Int*. **1992**, *24*, 285–307.

[24] Flessner, T.; Doye, S. Cesium Carbonate: A Powerful Inorganic Base in Organic Synthesis. *J. Prakt. Chem.* **1999**, *341*, 186–190. DOI: 10.1002/(SICI)1521-3897(199902)341:2<186::AID-PRAC186>3.0.CO;2-6.