Organic LETTERS

Letter

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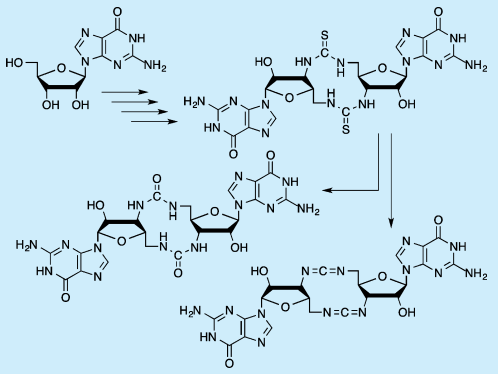
Synthesis of c-di-GMP Analogs with Thiourea, Urea, Carbodiimide, and Guanidinium Linkages

Barbara L. Gaffney and Roger A. Jones*

Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, 610 Taylor Road, Piscataway, New Jersey 08854, United States

Supporting Information

ABSTRACT: The first syntheses of neutral thiourea, urea, and carbodiimide analogs, along with two guanidinium analogs, of the bacterial signaling molecule cyclic diguanosine monophosphate (c-di-GMP) are reported. The key intermediate, obtained in nine steps, is a 3′-amino-5′-azido-3′,5′-dideoxy derivative. The 5′-azide serves as a masked amine from which the amine is obtained by Staudinger reduction, while the 3′-amine is converted to an isothiocyanate that, while stable to chromatography, and Staudinger conditions, nevertheless reacts well with the 5′-amine.



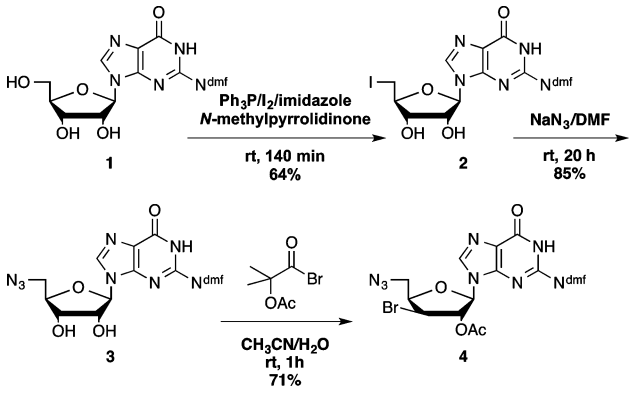
The bacterial signaling molecule cyclic diguanosine monophosphate (c-di-GMP) is responsible for regulating bacterial responses to a variety of environmental factors, including aggregation into the biofilm state.[1−6] Binding of c-di-GMP as a monomer and as a self-intercalated dimer to the PilZ domain proteins has been demonstrated.[1,2,7,8]

Activation of two different classes of riboswitches in noncoding regulatory mRNA domains also has been identified upon binding c-di-GMP.[9−12] Finally, c-di-GMP, among other cyclic dinucleotides, plays a role in triggering an innate immune response[13,14] through a transmembrane protein named STING in the innate immune sensing pathway, where a specific receptor for cyclic dinucleotides has been identified.[15]

A number of synthetic routes to c-di-GMP and its thiophosphate analogs have been reported.[16−19] Two analogs with a nonphosphate backbone have been prepared, one a methylphosphonate,[20] the other a carbamate,[21] but each lacks a 2′-hydroxyl group. An analog with a 2′-fluoro in place of the 2′-hydroxyl, with a phosphate backbone, was reported most recently.[22] The goal of the work reported below was to prepare c-di-GMP analogs with urea or urea related backbone linkages that should be stable to the bacterial phosphodiesterases that regulate c-di-GMP. The syntheses start with the introduction of nitrogen atoms to the guanosine 3′ and 5′ positions. The first steps are to prepare the 5′-azido-5′-deoxy derivative **3**, as shown in Scheme 1.

The N^2-dimethylformamidine (dmf) derivative of guanosine, **1**, was prepared by standard methods as described in detail in the Supporting Information. Preparation of **2** and **3** followed procedures reported for guanosine by Martin[23] and by Dean,[24] respectively. The major differences in this case were that heating was not required for the reaction of **2** with sodium azide and that **3** was readily isolated simply by the addition of methanol to the reaction mixture. The N^2-dmf group was used in this synthesis, as it has been shown to be essential for the reaction of guanosine with α-acetoxyisobutyryl bromide.[25] The reaction of **3** with this reagent proceeded analogously to that reported for **1**, with no degradation of the azido group under the acidic reaction conditions. In addition to the desired product, **4**, a small amount of the 2′-Br isomer was produced, in the ratio of 92:8, by LC-MS. These isomers were not separable by silica chromatography, but **4** was readily crystallized from methylene chloride, which efficiently removed the 2′-Br isomer. No chromatography was required for the preparation of compounds **1**−**4**, so that these reactions were conveniently

Scheme 1. Synthesis of Key Intermediate 4

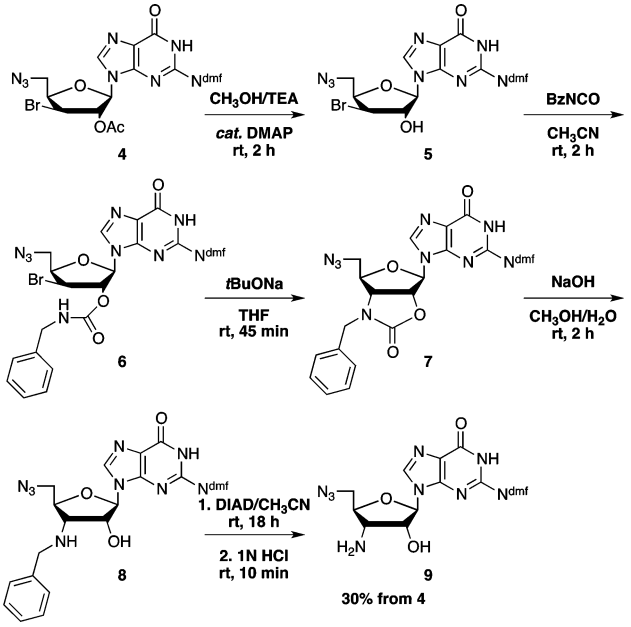


Received: November 1, 2013
Published: December 6, 2013

carried out starting with 20 g of guanosine to give **4** in an overall yield of 38%.

The conversion of **4** to the 3′-amino-5′-azido derivative **9**, shown in Scheme 2, proceeded analogously to the preparation

Scheme 2. Synthesis of 3′-Amino-5′-azido Derivative 9

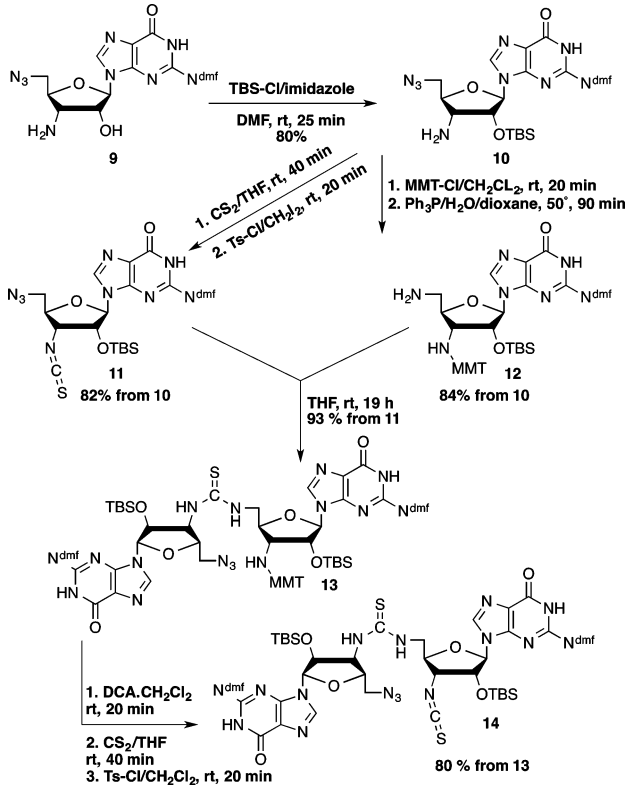


of 3′-amino-3′-deoxyguanosine reported by Zhang, although by using extensively altered conditions, and some different reagents, the reaction times were significantly reduced.[26] Catalytic DMAP in methanol with a few equivalents of TEA effected clean removal of the acetyl group from **4**. The reaction of **5** with benzylisocyanate in acetonitrile then proceeded in 2 h to give **6**. After investigating numerous reagents for cyclization to **7**, *t*BuONa in THF was found to give complete conversion in 45 min. Saponification of **7** to **8** by addition of 10 N NaOH to a methanol solution of **7** proceeded in 2 h. It is somewhat surprising that the N^2-dmf group survived these strongly basic conditions with only minimal loss. After neutralization of the reaction mixture, **8** was isolated by extraction. The steps from **4** to **8** were carried out in one flask, without isolation of intermediates, and **8** did not need purification before conversion to **9**.

Because of the 5′-azide it was not possible to use reduction to debenzylate the 3′-amino group in **8**, and instead oxidation using diisopropylazodicarboxylate (DIAD) was employed.[27] This is a slow reaction that required overnight to give the corresponding imine (not shown). Hydrolysis to **9** was effected using 1 N HCl, within 10 min, again with minimal loss of the N^2-dmf group. After neutralization of the reaction mixture with $NaHCO_3$, **9** was isolated by extraction, in this case remaining in the aqueous phase, while excess reagent was removed in the organic phase. The purification of **9** was carried out by reversed phase chromatography using 10 mM aqueous ammonium bicarbonate and acetonitrile, to give **9** in a yield of 30% from **4**. Although the N^2-dmf group survives limited time treatment with NaOH or HCl, it is slowly hydrolyzed by the ammonium bicarbonate eluant, so that solutions of **9** should not be allowed to stand for long periods of time after purification.

The derivatization of **9** for synthesis of the cyclic dimers required protection of the 2′-hydroxyl, conveniently done by reaction with *tert*-butyldimethylsilyl chloride, as shown in Scheme 3. Addition of the TBS group makes **10** again

Scheme 3. Synthesis of the Linear Dimer 14



amenable to silica chromatography, and all of the subsequent intermediates were purified on silica using gradients of methanol (with 0.5% TEA for those with a free amino group or the acid labile monomethoxytrityl group) and methylene chloride. The strategy for synthesis of the linear and cyclic dimers was to elaborate the 3′-amino group into an isothiocyanate and to couple this to a 5′-amino group obtained by Staudinger reduction of the 5′-azide. The 3′-isothiocyanate is stable to silica chromatography, so that intermediates **11** and **14** are easily handled, but it does react well with the 5′-amino group. Thus the 5′-azide functions as a stable masked amino group that can be converted to the amine without harming the 3′-isothiocyanate of **14**.

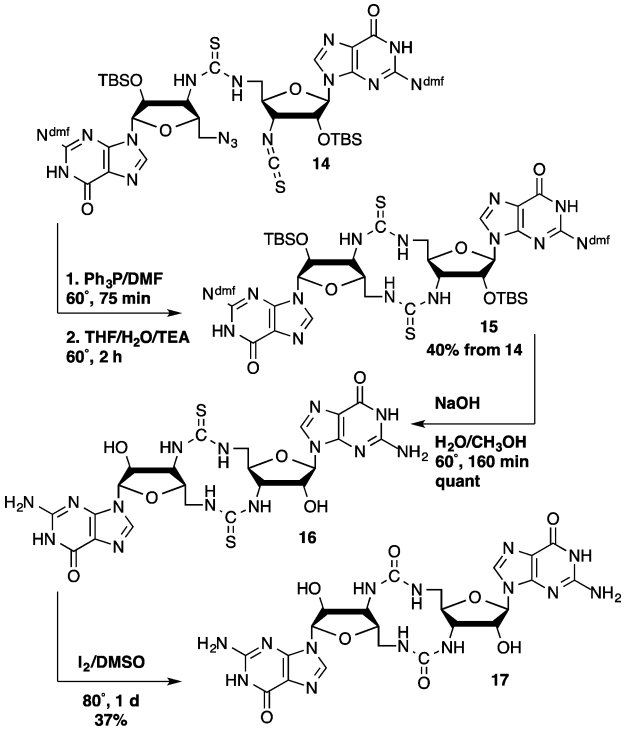
Formation of the 3′-isothiocyanate derivative **11** was carried out by reaction of **10** with carbon disulfide followed by reaction of the resulting dithiocarbamate (not shown) with tosyl chloride or benzenesulfonyl chloride.[28] This was done as a two-step procedure using a 10-fold excess of CS_2 in the first step that was readily removed on a rotary evaporator before reaction with the sulfonyl chloride. The 5′-amino nucleoside **12** was obtained by Staudinger reduction after protection of the 3′-amino group of **10** by reaction with monomethoxytrityl chloride. Condensation of **11** and **12** in THF at room temperature gave clean conversion to the linear dimer **13** within 17 h in 93% yield. The monomethoxytrityl group was removed using dichloroacetic acid (DCA), and the amino group converted to an isothiocyanate to give **14** by the same two-step procedure used for preparation of **11**.

Cyclization of the linear dimer **14** to the cyclic dimer **15** was effected by a two-step sequence starting with reaction of **14**

dx.doi.org/10.1021/ol403154w | Org. Lett. 2014, 16, 158−161

with triphenyl phosphine to give the azine (not shown, but sufficiently stable to be clearly visibly by LC-MS), followed by dilution of the reaction mixture with THF/water/TEA and heating at 60 °C for 2 h (Scheme 4). Although LC-MS showed

Scheme 4. Syntheses of Thiourea 16 and Urea 17

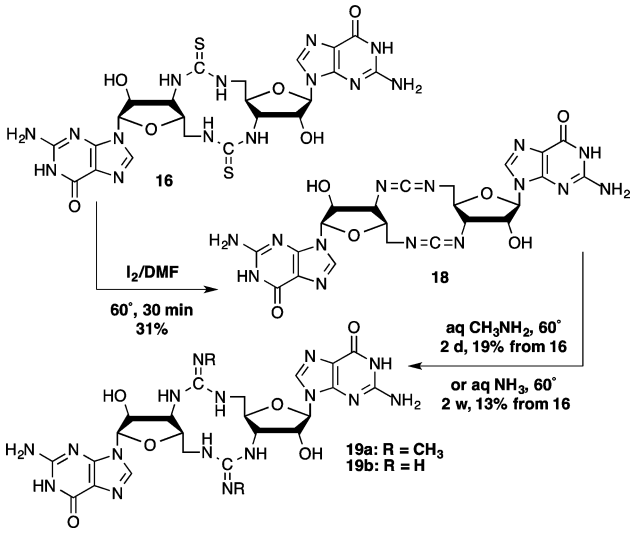


that the protected cyclic dimer **15** was the only significant product, there were a number of small impurities visible, possibly oligomers from an intermolecular reaction, even under the dilute conditions of the cyclization. It was again possible to purify **15** by silica chromatography, using a steep gradient of methanol in methylene chloride. The isolated yield for **15** was only 40%, presumably because of competing intermolecular reactions or degradation.

The deprotection of **15** to the cyclic thiourea **16** was effected using 2 N NaOH in methanol/water (1:1). Under these conditions the TBS groups were removed in minutes, at room temperature, while the N^2-dmf groups required heating at 60 °C for 2 h to effect removal, consistent with the surprising stability noted earlier. Neutralization of the reaction mixture with either 1 N HCl or acetic acid caused precipitation of **16**, which was isolated by filtration in quantitative yield. Of the many potential routes for conversion of thioureas to ureas,[29] reaction of **16** with DMSO and catalytic iodine, at 80 °C, was employed.[30] This is a simple, if slow, procedure that does not involve metals or unusual conditions and gave clean conversion to **17**, in 37% yield.

The reaction of **16** with iodine, this time in DMF at room temperature with triethylamine, was also effective for preparation of the carbodiimide **18** (Scheme 5).[31] Although this reaction is reported to require aryl thioureas,[31] it worked well for preparation of **18**. The reaction of carbodiimides with amines for synthesis of guanidines is well-known,[32] and aqueous methylamine and aqueous ammonia gave **19a** and **19b**, respectively, although slowly and in modest yields.

Scheme 5. Syntheses of Carbodiimide 18 and Guanidines 19a and 19b



The carbodiimide **18** proved to be sufficiently stable to be handled and purified using the same conditions used for **17** and **19a/b**. All of these compounds have poor solubility in water, but are soluble in 0.1 N NaOH. Purification of each was done by RP chromatography using 0.1 N NaOH and methanol. Neutralization of the product fractions using CO_2 gas gave each compound as a white solid easily isolated by filtration. The preparations of **17**, **18**, and **19a/b** were carried out on small scales only and were not optimized.

Recent reports of activation of the innate immune system with the 2′/3′ isomers of cyclic GMP-AMP (cGAMP)[33−35] provide a new impetus for preparation of cyclic dinucleotides (CDNs) and their analogs. The compounds reported here are the first examples of a new class of CDN analogs that possess a urea, or urea related, backbone.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, HPLC, ^{1}H, ^{13}C NMR, and UV spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: r.jones@rutgers.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was initated with support from NIH Grant GM79760.

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