Bioorganic & Medicinal Chemistry Letters 18 (2008) 5631–5634

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and immunostimulatory properties of the phosphorothioate analogues of cdiGMP

Hongbin Yan [a,*], Xiaolu Wang [a], Rhonda KuoLee [b], Wangxue Chen [b]

[a] Department of Chemistry, Brock University, 500 Glenridge Avenue, St. Catharines, Ont., Canada L2S 3A1
[b] Institute for Biological Sciences, National Research Council Canada, Ottawa, Ont., Canada K1A 0R6

ARTICLE INFO

Article history:
Received 24 July 2008
Revised 23 August 2008
Accepted 26 August 2008
Available online 29 August 2008

Keywords:
cdiGMP
Vaccine adjuvant
Immunostimulation
Phosphorothioate

ABSTRACT

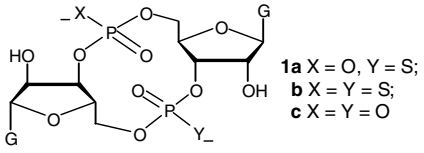
The synthesis of mono- and bisphosphorothioate analogues of 3′,5′-cyclic diguanylic acid (cdiGMP) via the modified H-phosphonate chemistry is reported. The immunostimulatory properties of these analogues were compared with those of cdiGMP.

3′,5′-Cyclic diguanylic acid (cdiGMP, **1c**) has recently been recognized as an important bacterial second messenger.[1–3] It has also been shown to possess extraordinary immunostimulatory properties and is therefore evaluated as a potential vaccine adjuvant candidate.[4–7]

We previously reported a convenient synthesis of cdiGMP.[8] In order to explore the structure–immunostimulation relationship of cdiGMP, we synthesized the phosphorothioate analogues of cdiGMP (Fig. 1), where either one (cdiGMP-S1 **1a**)[9] or two (cdiGMP-S2 **1b**) sulfur atoms replace the non-bridging oxygen at the internucleotide linkages.

The 2′- and 5′-hydroxyls of guanosine were protected with the 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl (Cpep)[10] and the 9-phenyl-xanthen-9-yl (or the pixyl)[11,12] groups, respectively (Fig. 2). Guanine was 'doubly'-protected at both *O*-6 and *N*-2, as is shown in Figure 2. The modified H-phosphonate approach[13,14] was used due to its flexibility in the preparation of both phosphates and phosphorothioates.

The synthesis of the phosphorothioates via the modified H-phosphonate approach is illustrated in Scheme 1. In situ treatment of H-phosphonate diesters with a sulfur-transfer reagent *S*-(2-cyanoethyl)phthalimide **9** gave phosphorothioate triester **4**, which was further transformed into linear dimer H-phosphonate **6**. Cyclization of this linear dimer H-phosphonate **6** took place under high dilution conditions to furnish the fully protected cyclic dinucleotide phosphorothioate trieters **7a** and **7b** in good yields (75–80%).



1a X = O, Y = S;
b X = Y = S;
c X = Y = O

Figure 1. cdiGMP **1c** and its phosphorothioate analogues.

A four-step deprotection protocol (Scheme 2) was used to give the fully deprotected cdiGMP-S1 **1a** and cdiGMP-S2 **1b** in good yields (70–75%).[15] Removal of the *S*-(2-cyanoethyl)- group by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under anhydrous conditions was followed by treatment with 2-nitrobenzaldoxime **12** and ammonolysis in the presence of mercaptoethanol (Scheme 2, steps i–iii). The resulting partially protected cyclic dimers **11** were then further deprotected in a triethylammonium formate buffer that contains methanol (Scheme 2, steps iv and v). The ^{1}H and ^{31}P NMR spectra of **1a** and **1b** are shown in Figure 3. Resonance at ca. 55 and −1 ppm in the ^{31}P NMR spectra correspond to phosphorothioate and phosphate, respectively. It is noted that the two sets of phosphorous signals in cdiGMP-S1 **1a** integrate equally (panel b) and that there is no signal at ca. 0 ppm in the cdiGMP-S2 **1b** (panel d).

The fully deprotected cdiGMP-S1 **1a** and S2 **1b** were also analyzed by reverse phase HPLC on a Dionex Acclaim PA C_{18} column (Fig. 4).

We then carried out preliminary evaluation of the immunostimulatory properties of cdiGMP, cdiGMP-S1, and cdiGMP-S2. In

* Corresponding author. Tel.: +1 905 688 5550x3545; fax: +1 905 682 9020.
E-mail address: tyan@brocku.ca (H. Yan).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.08.088

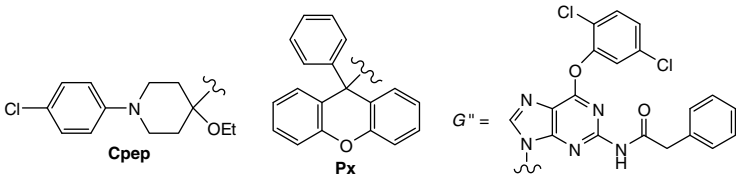


Figure 2. Protecting groups used in this study.

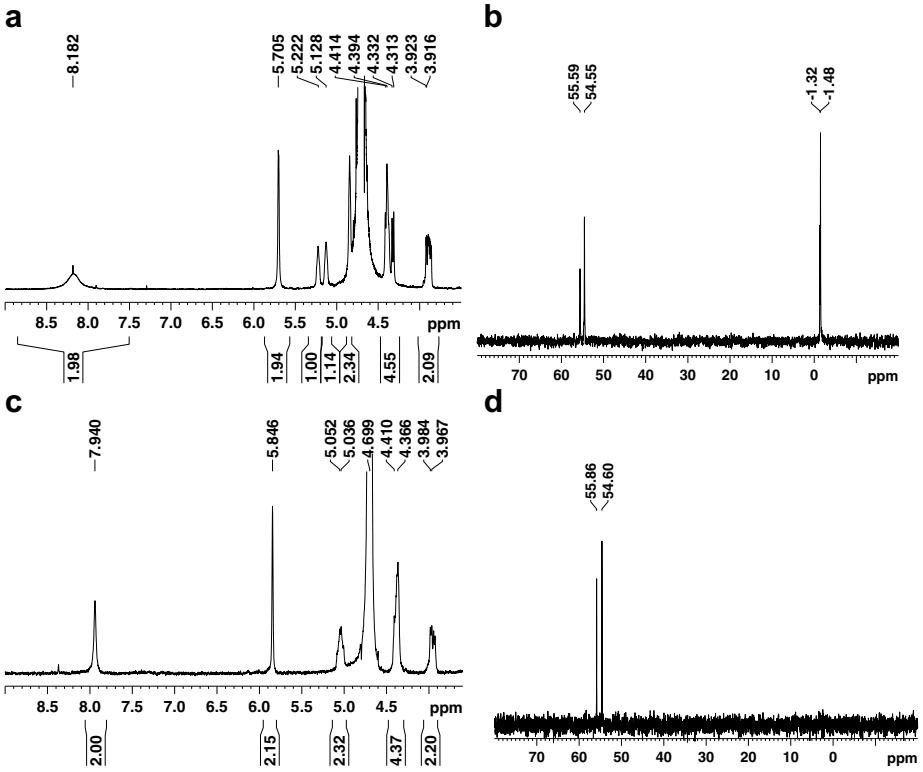
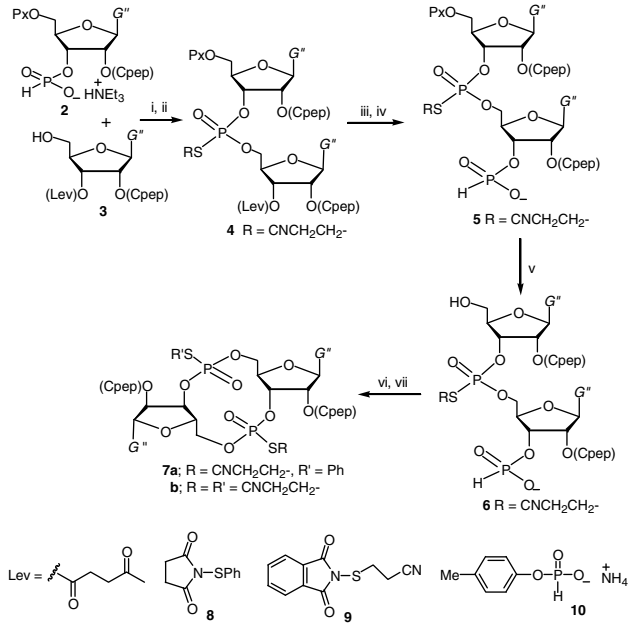
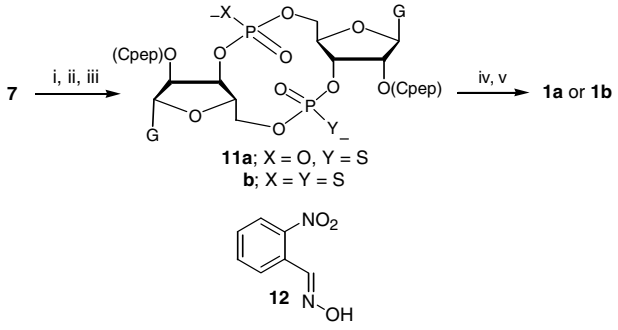


Figure 3. ^{1}H and ^{31}P NMR spectra of **1a** (a and b) and **1b** (c and d).



Scheme 1. Synthesis of fully protected cdiGMP-S1 and S2. (i) $(CH_3)_3COCl$, C_5H_5N; (ii) **9**, C_5H_5N; (iii) $NH_2NH_2{\cdot}H_2O$, CH_3COOH, H_2O, C_5H_5N; (iv) **10**, $(CH_3)_3COCl$, C_5H_5N, 0 °C; (v) CF_3COOH, pyrrole, CH_2Cl_2; (vi) $(PhO)_2P(O)Cl$, CH_2Cl_2, C_5H_5N, −40 °C; (vii) **8** or **9**, C_5H_5N.

the first experiment, groups of five female 8-week-old C57BL/6 mice were intranasally administered with 0, 5, 10, and 69 μg of cdiGMP in injectable phosphate buffered saline (PBS). The mice were killed 24 h later and their lungs were lavaged with PBS supplemented with 3 mM EDTA (1 ml, 5×). The total and differential cell counts as well as a panel of 21 chemokines and cytokines were measured. As can be seen in Figure 5, intranasal instillation of cdiGMP induced a dose-dependent recruitment of inflammatory cells into the bronchoalveolar spaces with the majority of recruited



Scheme 2. Unblocking of fully protected cdiGMP-S1 and S2. (i) DBU, $(CH_3)_3SiCl$, CH_3CN; (ii) **12**, DBU, CH_3CN; (iii) aq NH_3, $HSCH_2CH_2OH$, 55 °C; (iv) CH_3OH, NEt_3-HCOOH buffer (pH 3.75), 40 °C, 4 h; (v) Amberlite IR-120, Na^+ form.