Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Organometallic Chemistry 680 (2003) 124–126
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Synthesis of adenosine containing carborane modification

Agnieszka B. Olejniczak [a], Andrey Semenuk [b], Marek Kwiatkowski [b], Zbigniew J. Lesnikowski [a,*]

[a] *Laboratory of Molecular Virology and Biological Chemistry, Center for Microbiology and Virology PAS, 106 Lodowa St., Lodz 93-232, Poland*
[b] *Institutionen för Genetik och Patologi, Rudbecklaboratoriet, Uppsala Universitet, Uppsala 751 85, Sweden*

Received 3 March 2003; received in revised form 25 March 2003; accepted 26 March 2003

This paper is dedicated to Professor Frederick M. Hawthorne on the occasion of his 75th birthday

Abstract

The carboranyl cage is a new modifying entity for nucleosides and DNA-oligonucleotides. Most of carborane–nucleoside conjugates described so far belong to pyrimidine series. Herein, the first synthesis of adenosine, nucleoside containing purine nucleic base, modified with carborane cluster, is described.

Keywords: Boron; Carborane; Nucleosides; Antiviral; BNCT

1. Introduction

The original rationale for the design and synthesis of boron-containing nucleosides is their potential application as boron rich carriers for boron neutron capture therapy (BNCT) [1,2]. In addition, boron cage is being pursued recently as lipophilic pharmacophore for antiviral nucleosides and other biomolecules [2,3]. Most of carborane modified nucleosides belong to pyrimidine series. Despite the fact that purine nucleosides such as adenosine and guanosine play an important role in cellular metabolism this class of carborane modified nucleosides focused less attention due to difficulties in their preparation. For example, synthesis of a carborane-containing purine nucleoside inosine was attempted, but the 2-*ortho*-carboranyl-inosine precursor could not be deprotected [4,5].

Adenosine is an endogenous modulator of intercellular signaling that provides homeostatic reduction in cell excitability during tissue stress and trauma [6]. The adenine nucleoside phosphates: AMP (adenosine 5′-monophosphate), ADP (adenosine 5′-diphosphate), ATP (adenosine 5′-triphosphate), that are maintained in equilibrium by adenylate kinase (AK), constitute the bulk of the purine nucleotide pool. In addition, ATP is the most important molecule for capturing and transferring free energy in most organisms and it is a substrate for RNA polymerases. Another adenosine phosphate cAMP (cyclic adenosine 3′,5′-monophosphate) is the second messenger for many hormones and plays important role in the regulation of cellular metabolism.

Availability of the methods for the synthesis of adenosine conjugates bearing boron rich, lipophilic carborane cluster seems therefore highly desirable and will provide an opening for the synthesis and study of adenosine and its biologically important phosphate analogues containing carborane cage. In this communication we present the first synthesis of adenosine with carborane modification attached to sugar residue at 2′-position.

As the modifying entity *para*-carborane cage was chosen. Though, *ortho*-carborane derivatives are easily available and less expensive than *para*-counterparts, the closed *ortho*-carborane cage decomposes in basic conditions into its open cage, *nido*-form which is often associated with significantly increased toxicity.

* Corresponding author. Tel.: +48-42-6771249; fax: +48-42-6771230.
E-mail address: zlesnik@cmiwpan.lodz.pl (Z.J. Lesnikowski).

0022-328X/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0022-328X(03)00217-1

2. Results and discussion

The successful approach to the synthesis of 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (2′-CBA, **4**) is based on the nucleophilic substitution of activated thiomethyl group in 6-*N*-benzoyl -3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methylenethiomethyladenosine (**1**) with suitable alcohol bearing carborane cage [7] (Fig. 1). The key intermediate **1** was obtained in the reaction of 6-*N*-benzoyl-3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine with DMSO in a mixture of acetic acid–acetic anhydride 9.

The 6-*N*-benzoyl-3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine, precursor for the synthesis of **1** is easily available and can be prepared with high yield according to the literature procedure from adenosine (A) [8]. Thus, first the amino group of adenine in position 6 is protected using benzoyl chloride in pyridine [10] then 3′- and 5′-hydroxyl functions are protected in the reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane [11]. The same method is routinely applied for the synthesis of analogous derivatives of three other common ribonucleosides: uridine (U), cytidine (C) and guanosine (G).

Target compound **4** was obtained in three-step procedure. In the first step, 1-(3-hydroxypropyl)-*para*-carborane (**5**) was reacted with **1** yielding fully protected 6-*N*-benzoyl-3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**2**) (Fig. 1). Next, the disiloxane protecting group in **2** was removed with TBAF in THF solution yielding 6-*N*-benzoyl-2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**3**). The benzoyl protection in **3** was removed with concentrated aqueous ammonia solution providing 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**4**) (Fig. 2). Compound **4** was characterized using chromatographic and instrumental methods.

It should be pointed out that the proposed approach forms a base for the general method for the synthesis of

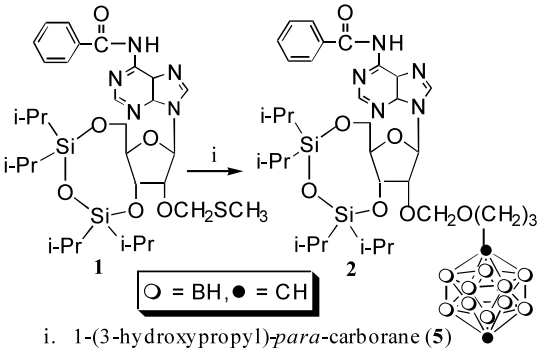

Fig. 1. Synthesis of 6-*N*-benzoyl-3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**2**).

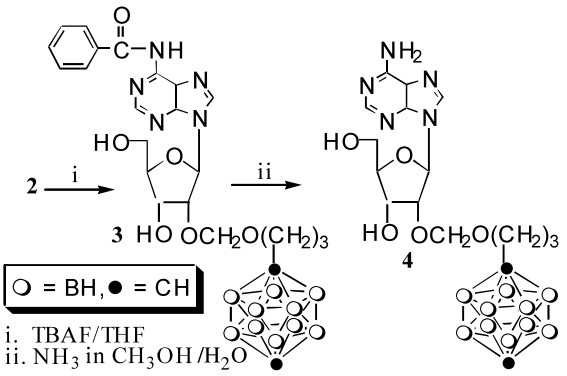

Fig. 2. Synthesis of 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**4**).

nucleosides bearing carborane cage at 2′-position. It is also an alternative to the literature procedure based on the reaction of alkynyl nucleoside derivatives and decaborane complex [12] and can provide a route to nucleoside conjugates modified with different types of carborane cages as long as suitable alcohol terminated with carborane cluster is available. The work in this direction as well as on the synthesis of adenosine phosphates containing carborane modification is in progress in our laboratory.

3. Experimental

3.1. Materials

Adenosine was purchased from Avocado Lancaster (Mühlheim am Main, Germany). Column chromatography was performed on silica gel 230–400 mesh obtained from Sigma–Aldrich (Steinheim, Germany). TLC was performed on silica gel F254 plates purchased from Sigma–Aldrich (Steinheim, Germany). Solvents were purchased in the highest available quality. UV measurements were performed on GBC Cintra10e UV–vis spectrometer (Dandenong, Australia). ^{1}H- and ^{11}B-NMR spectra were recorded on a Bruker Avance DPX 250 spectrometer equipped with BB inverse probe-head operating at 250.13 and 81.21 MHz, respectively. Tetramethylsilane and external $BF_3/(C_2H_5)_2O$ were used as standards for ^{1}H- and ^{11}B-NMR, respectively. Fast atom bombardment (FAB, Gly) mass spectra were recorded on a Finnigan MAT spectrometer (Bremen, Germany).

3.2. Methods

3.2.1. 1-(3-Hydroxypropyl)-para-carborane (5)

Compound **5** was obtained according to the literature procedure [13].

*3.2.2. 6-N-Benzoyl-2′-O-[(para-carboran-1-yl)propyleneoxymethyl]adenosine (**3**)*

Compound **3** was obtained in the reaction of 6-*N*-benzoyl-3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methylenethiomethyladenosine (**1**, 50 mg, 0.07 mmol) with 1-(3-hydroxypropyl)-*para*-carborane (**5**, 37 mg, 0.19 mmol) at the presence of copper(II) bromide and tetrabutylammonium bromide as activators; as the reaction medium 1,2 dichloroethane was used. The reaction progress was monitored by TLC (CH_2Cl_2–CH_3OH, 9:1). After reaction completion (ca. 50 h at room temperature (r.t.)) solvent was evaporated under reduced pressure, then the oily residue was dissolved in dichloromethane (5 ml). The resultant solution was washed with water (3×3 ml) then the organic fraction was dried over anhydrous magnesium sulfate and evaporated to dryness under vacuum. The yield of crude product **2** was 120 mg (60%). Next, **2** without purification was dissolved in THF (1.5 ml) then TBAF (1 ml, 3.5 mmol) was added. After 15 min to the reaction mixture a pyridine–methyl alcohol–water (3:1:1, 2.5 ml) followed by ion exchange resin Dowex 50Wx8 in pyridinium form, was added. After 30 min the ion exchange resin was filtered off and washed with pyridine–methyl alcohol–water (3×5 ml). The filtrate and washings were combined together then whole evaporated to dryness under vacuum yielding crude **3**. Crude product **3** was purified by silica gel column chromatography (5 g, 230–400 mesh) using a linear gradient of MeOH in methylene chloride as a eluting solvent system (0–5%). Yield: 12 mg (30%). TLC (CH_2Cl_2–MeOH, 9:1): $R_f = 0.59$; UV (96% C_2H_5OH): $\lambda_{min} = 257.34$, $\lambda_{max} = 281.15$ nm; δ ^{1}H-NMR ($CDCl_3$): 1.34–1.48 (m, 2H, 2H–β-CH_2), 1.66–1.78 (m, 2H, 2H–γ-CH_2), 2.64 (bs, 1H, 1H–C-carborane), 3.50 (t, 2H, 2H–α-CH_2), 3.63–4.63 (m, 5H, 1H-2′, 1H-3′, 1H-4′, 2H-5′), 4.96 (m, 2H, OCH_2), 6.00 (s, 1H, 1H-1′), 7.51–7.73 (m, 5H, of benzoyl group), 8.05 (s, 1H, 1H-2), 8.80 (s, 1H, 1H-8); δ ^{11}B{^{1}H}-NMR ($CDCl_3$): −12.50 (s, 5B), −15.01 (s, 5B); FAB-MS (+VE, Gly) 587.3 [M^+] (molecular formula: $C_{24}H_{41}B_{10}N_5O_5$, calculated exact mass = 587.2).

*3.2.3. 2′-O-[(para-Carboran-1-yl)propyleneoxymethyl]adenosine (**4**)*

The 6-*N*-benzoyl-2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]adenosine (**3**, 30 mg, 0.05 mmol) was dissolved in MeCN (0.2 ml) then concentrated aq. ammonia solution was added (2 M, 2 ml). After 2 h at r.t. (TLC control, CH_2CL_2–MeOH, 9:1) solvents were evaporated under vacuum then crude **4** was purified by means of semi-preparative TLC using CH_2Cl_2–MeOH, 9:1 as a eluting solvent system. Yield: 23 mg (93%). TLC (CH_2Cl_2–MeOH, 9:1): $R_f = 0.26$; UV (96% C_2H_5OH): $\lambda_{min} = 239.60$, 298.57, $\lambda_{max} = 256.88$ nm, 313.60 nm; δ ^{1}H-NMR (CD_3OD): 0.97–1.10 (m, 2H, -CH_2), 1.34–1.41 (m, 2H, γ-CH_2), 2.41 (bs, 1H, 1H-$_{C\text{-carborane}}$), 2.94–3.08 (m, 2H, a-CH_2), 3.75–3.90 (dd, 2H, 2H-5′), 4.15–4.20 (d, 1H, 1H-4′), 4.35–4.45 (m, 1H, 1H-3′), 4.50–4.60 (t, 2H, OCH_2), 4.65–4.70 (d, 1H, 1H-2′), 6.04 (d, 1H, 1H-1′), 8.20 (s, 1H, 1H-2), 8.29 (s, 1H, 1H-8); δ ^{11}B{^{1}H}-NMR (CD_3OD): −10.23 (s, 5B), −12.67 (s, 5B). FAB-MS (+VE, Gly) 483.4 [M^+] (molecular formula: $C_{17}H_{37}B_{10}N_5O_4$, exact mass: 483.2).

Acknowledgements

This work was supported by the Polish Committee for Scientific Research (KBN), grant 6 PO5F 023 20p02. We thank Professor Stefan Sjöberg (Uppsala University) for generous gift of *para*-carborane and his assistance and helpful discussions during the early stages of this project.

References

[1] M.F. Hawthorne, Angew. Chem. Int. Ed. Engl. 32 (1993) 950.
[2] Z.J. Lesnikowski, J. Shi, R.F Schinazi, J. Organomet. Chem. 581 (1999) 156.
[3] K. Yamamoto, Y. Endo, Bioorg. Med. Chem. Lett. 11 (2001) 2389.
[4] Y. Yamamoto, T. Seko, H. Nakamura, Heteroat. Chem. 3 (1992) 239.
[5] Z.J. Lesnikowski, R.F. Schinazi, Pol. J. Chem. 69 (1995) 827.
[6] E.A. Kowaluk, S.S. Bhagwat, M.F. Jarvis, Curr. Pharm. Design 4 (1998) 403.
[7] M. Matteuci, K-Y. Lin, S. Butcher, C. Moulds, J. Am. Chem. Soc. 113 (1991) 7767.
[8] B.S. Sproat, Methods in molecular biology, in: S. Agrawal (Ed.), Protocols for Oligonucleotide Analogues. Synthesis and Properties, vol. 20, Humana Press Inc, Totowa, NJ, 1993, pp. 115–141.
[9] (a) J. Hovinen, E. Azhayeva, A. Azhayev, A. Guzaev, H. Loonnberg, J. Chem. Soc. Perkin Trans. I (1994) 211;
(b) D. Sawada, Y. Ito, Tetrahedron Lett. 42 (2001) 2501.
[10] G.S. Ti, B.L. Gaffney, R.A. Jones, J. Am. Chem. Soc. 104 (1982) 1316.
[11] W.T. Markiewicz, J. Chem. Res., (S) 24 (1979) 25.
[12] W. Tjarks, A.K.M. Anisuzzaman, L. Liu, A.H. Soloway, R.F. Barth, D.J. Perkins, D.A. Adams, J. Med. Chem. 35 (1992) 1628.
[13] J. Malmquist, S. Sjöberg, Tetrahedron 52 (1996) 9207.