Journal of Organometallic Chemistry 694 (2009) 1771–1775

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

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

Nucleoside–boron cluster conjugates – Beyond pyrimidine nucleosides and carboranes

Zbigniew J. Lesnikowski *

Institute of Medical Biology, Laboratory of Molecular Virology and Biological Chemistry, Polish Academy of Sciences, 106 Lodowa St., 93-232 Lodz, Poland

ARTICLE INFO

Article history:
Received 18 November 2008
Received in revised form 22 December 2008
Accepted 24 December 2008
Available online 3 January 2009

Keywords:
Boron clusters
Nucleosides
BNCT
Antivirals
Pharmacophors
Bioinorganic materials

ABSTRACT

General methods for the synthesis of new purine and pyrimidine nucleosides modified with borane clusters and metallacarborane complexes are presented. They include: (1) attachment of carborane modification at 2′ position of nucleoside *via* formacetal linkage formation, (2) tethering of the metallacarborane group at nucleobase part of the nucleoside *via* dioxane ring opening in oxonium metallacarborane, carborane or dodecaborate derivatives, and (3) "click" chemistry approach based on Huisgen 1,3-dipolar cycloaddition. Proposed methodologies extend the range of nucleoside–boron cluster conjugates available and open new areas for their applications.

1. Introduction

Due to their essential role in virtually all cellular processes nucleosides are one of the most important small biomolecules. They are basic building blocks of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), play many important functions as themselves, in phosphorylated form (nucleotides) or in conjugation with other biomolecules [1]. Because their crucial role in many metabolic pathways and interactions with other biomolecules, nucleosides, their derivatives and analogues are widely used as chemotherapeutics, mainly as antiviral and anticancer agents [2,3].

Boron containing nucleosides were originally designed as potential boron carriers for boron neutron capture therapy (BNCT) of tumors [4,5]. As boron rich donors in boron carrying molecules dicarba-*closo*-dodecaboranes ($C_2B_{10}H_{12}$) are frequently used due to their chemical and biological stability and physicochemical versatility [6]. The rationale for the synthesis and study of nucleoside derivatives as boron carriers for BNCT is that nucleoside–boron cluster conjugates may be selectively accumulated in rapidly multiplying tumor cells, and following their phosphorylation converted to the corresponding nucleotide, trapped within the cell or ideally, incorporated into nuclear DNA of tumors [7,8].

Another major class of nucleoside–boron cluster conjugates are nucleoside based antivirals [6]. E.g., the superior antiviral profiles of (−)-β-L-2′,3′-dideoxy-3′-thiacytidine (3TC) and (−)-β-L-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC) encouraged introduction of oxathialane heterocycle to the carborane-containing nucleosides [9,10]. Since 2′-fluoronucleosides exhibited broad anti-herpes and anti-hepadnaviruses activity, 2′-deoxy-2′-fluoroarabinose nucleosides containing carboranyl group such as 5-*ortho*-carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinosyl)uracil (CFAU) were synthesized [11]. Demonstrated by β-D-2′,3′-didehydro-2′,3′-dideoxythymidine (D4T) and β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (D-D4FC) potent antiviral activity against HIV and HBV prompted synthesis of series of 2′,3′-didehydro-2′,3′-dideoxy-nucleosides (D4N) containing carborane such as 5-*ortho*-carboranyl-2′,3′-didehydro-2′,3′-dideoxyuridine (D4CU: β-D, α-D, β-L, and α-L) [12].

It should be pointed out that in both cases of nucleoside–boron cluster conjugate applications: as boron carriers for BNCT and as boron containing antivirals, the nucleoside being a part of the conjugate belongs to the pyrimidine series of nucleosides. 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. This limitation can be explained by: (1) result of focus on modified nucleoside's phosphorylation, considered as prerequisite of expected biological activity, (2) lack of methods for synthesis of purine nucleoside–boron cluster conjugates. The first factor can be rationalized by higher abundance of kinase activities involved in pyrimidine nucleoside phosphorylation in human cells as compared to kinases specific for purine nucleosides [13]; the second factor is self-explanatory since till recently synthesis of purine nucleoside–boron cluster conjugates, with few exceptions, was neglected. For

* Tel.: +48 42 2723629; fax: +48 42 2723630.
E-mail address: zlesnikowski@cbm.pan.pl

0022-328X/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2008.12.061

example, synthesis of a carborane-containing purine nucleoside inosine was attempted, but the 2-*ortho*-carboranyl-inosine precursor could not be deprotected [14].

Other, general factor limiting applications of nucleoside–boron cluster conjugates involves lack of variety among the boron clusters used for nucleoside modification. By far most approaches utilized the *ortho*-carborane cage and neglected potential of the metallacarboranes and dodecaborates as modifying units for nucleosides [15]. The need for new, efficient methods of preparation of various nucleoside–boron cluster conjugates is additionally prompted by emerging new applications of these compounds. The boron clusters tethered to nucleosides are used as modifying entities for DNA-oligonucleotides potentially useful as antisense agents for antisense oligonucleotide therapy (AOT), as molecular probes for molecular diagnostics based on hybridization technology [16,17], as lipophilic pharmacophors [18–20], electrochemical [21,22] and infrared labels [23], and others [24].

Recent approaches to bypass above limitations and expand the number of methods for nucleoside–boron cluster conjugates synthesis will be discussed.

2. Modification of nucleosides with carborane cluster at 2′ position *via* formacetal linkage formation

The method for the synthesis of nucleosides bearing carborane cluster at 2′ position [25] is based on the formacetal linkage formation described by Matteucci et al. [26] and modified by Sawada and Ito [27]. It utilizes nucleophilic substitution of the activated methylthiomethyl group in fully protected 3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methylthiomethylnucleosides (**2a**–**d**) with a suitable alcohol bearing carborane cage (Scheme 1). Target compounds **3a**–**d** are obtained from intermediate **2a**–**d** in a three-step procedure without isolation and purification of the intermediate products. First, compounds **2a**–**d** are reacted with 1-(3-hydroxypropyl)-*para*-carborane (**1**) [28] yielding fully protected 3′,5′-*O*,*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]-nucleosides, then the disiloxane protection is removed using a solution of TBAF in THF yielding *N*-protected 2′-*O*-[(*para*-carboran-1-yl)propyleneoxymethyl]nucleoside. The acyl protections in **2b**–**d** is then removed with concentrated aqueous ammonia solution providing carborane containing conjugates, derivatives of all four canonical ribonucleosides: uridine (U), cytidine (C), adenosine (A) and guanosine (G), **3a**–**d**.

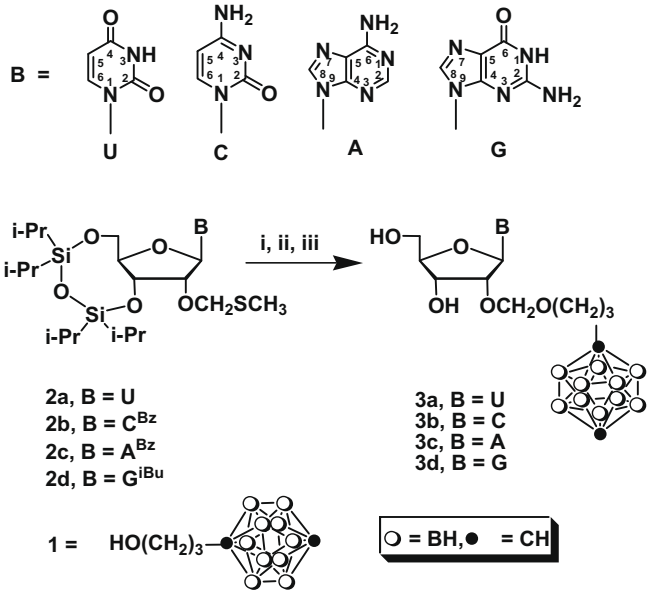
3. Nucleoside–metallacarborane conjugates

Metallacarboranes are presently focusing renewed attention for their anti-viral [29] and anti-tumor activities [30,31]. It is feasible that also conjugates of nucleosides and metallacarboranes could exhibit useful biological characteristics. Another advantage of nucleoside–metallacarboranes is their application as versatile synthons for synthesis of metal bearing DNA-oligomers for various applications.

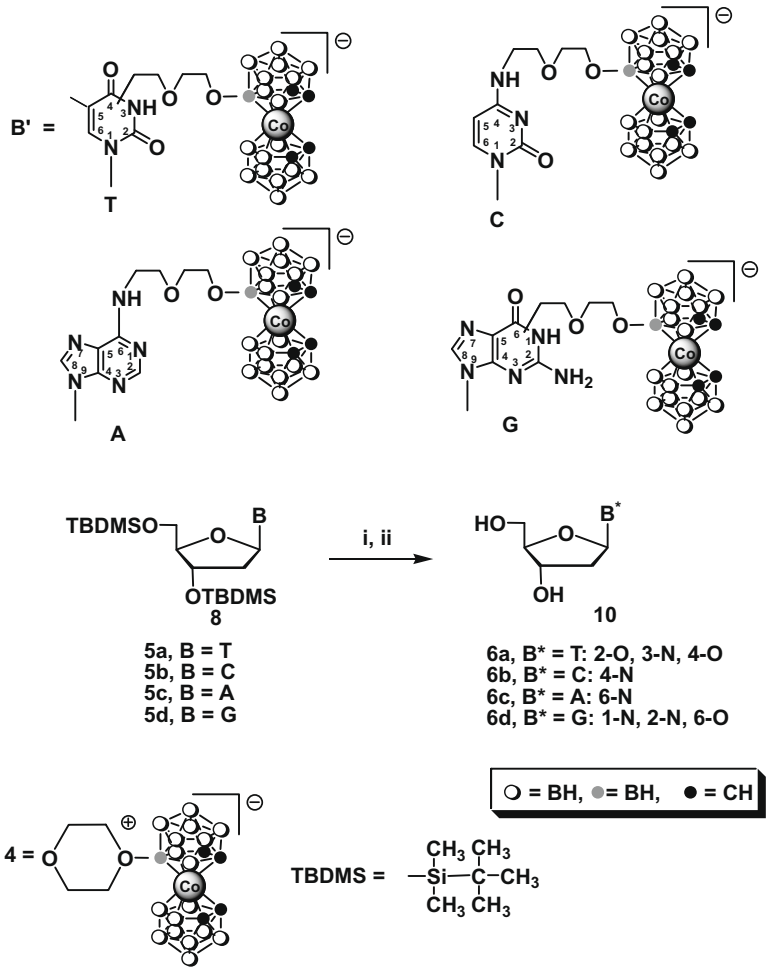
Metallacarboranes can potentially function as electrochemical and photo luminescent labels for nucleic acids [21], infrared labels [23], radioactive metal isotope carriers, active centers of DNA-directed artificial chemical nucleases, metal bearing components in construction of probes for DNA-mediated electron transfer, and others [32].

We proposed a general approach to the synthesis of nucleoside conjugates, derivatives of all four canonical deoxynucleosides: thymidine (T), 2′-deoxycytidine (dC), 2′-deoxyadenosine (dA) and 2′-deoxyguanosine (dG), containing metallacarborane complex [33]. Nucleoside–metallacarborane conjugates derivatives have been prepared in the reaction of ring opening in dioxane–metallacarborane adduct [34] by base activated 3′,5′-protected nucleoside.

The target nucleoside–metallacarborane conjugates were obtained in a simple, three-step procedure (Scheme 2). First, 5′- and 3′-hydroxyl functions of the nucleosides were protected with *tert*-butyldimethylsilyl group [35]. In the second step, each of 3′,5′-protected nucleosides **5a**–**d** was activated with an excess of sodium hydride then treated with dioxane–metallacarborane adduct **4** in anhydrous toluene as reaction medium. In the third step, the *tert*-butyldimethylsilyl protections were removed with TBAF in THF providing nucleoside–metallacarborane conjugates **6a–d**



Scheme 1. General method for the synthesis of nucleosides modified with boron cluster at 2′ position.



Scheme 2. General method for the synthesis of nucleosides modified with metallacarborane at nucleobase.

containing 5-[3-cobalt bis (1,2-dicarbollide)-8-yl]-3-oxa-pentoxy-modification at different locations within the nucleic acid base.

The proposed approach provides a route to nucleoside conjugates modified with metallacarboranes bearing not only cobalt as shown above but also different metals and different types of carborane cages as long as suitable adducts of the cyclic ether and boron cluster is available. Indeed, nucleoside-metallacarborane conjugates bearing iron [36,37], chromium [38] or rhenium [15] have been also obtained.

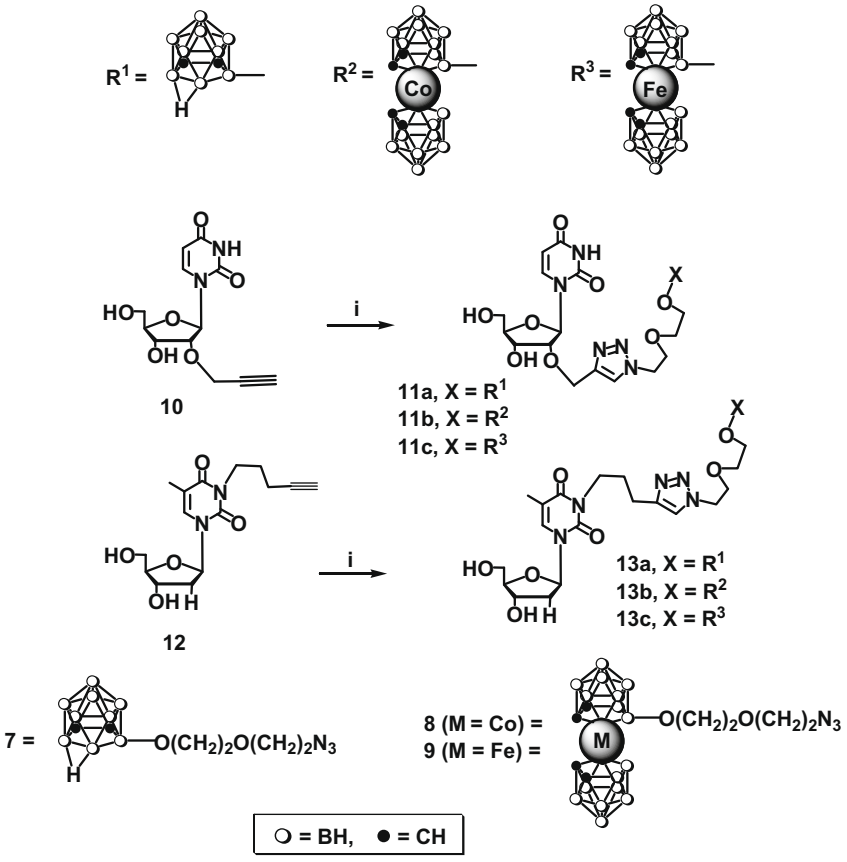
4. "Click"-chemistry – A general and versatile method for modification of nucleosides with boron clusters

The Cu(I)-catalyzed 1,3-dipolar cycloaddition of azide and alkyne to form a triazole, termed click chemistry, has been recently established as an important tool for chemical modification of biomolecules [39]. The 1,2,3-triazole functions are rigid linking unit that can mimic the atom placement and electronic properties of a peptide bond without the same susceptibility to hydrolytic cleavage. The reactants, alkyne and azide, are convenient to introduce independently, stable, and do not react with common organic reagents or functional groups in biomolecules (are orthogonal). All these factors allow broad application of click chemistry approaches in chemical synthesis of various classes of compounds. Recently we proposed a new and versatile approach based on Huisgen type cycloaddition [40] for modification pyrimidine as well as purine nucleosides with various boron clusters [41].

The target nucleoside–boron cluster conjugates **11a–c** and **13a–c** were obtained in a simple, one-step procedure (Scheme 3). The reaction was performed under standard click chemistry version of Huisgen azide-alkyne cycloaddition [42]. Suitable nucleoside acceptor with a spacer of different type and length terminated with ethyne or azide group (not shown) was dissolved in a mixture of *tert*-butanol and water, together with equimolar amount of boron cluster donor equipped with a 3-oxa-pentoxy-spacer terminated with azido or 2-propyn-1-oxy- or 4-pentyn-1-oxy-substituent (not shown). To the obtained solution a catalytic amount of $CuSO_4$ and potassium ascorbate solutions were added. Reactions were performed at room temperature during 8–50 h (usually 24 h) with a TLC control. After reaction completion the solvents were evaporated and the crude product was purified by silica gel column chromatography. The yield of purified product ranged usually from 30% to 65% [43,44].

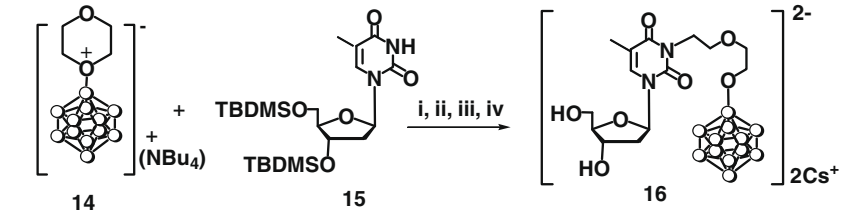
5. Dodecaborate-containing nucleoside conjugates

The high hydrophobicity of carborane unit often used for nucleoside modification causes low solubility of nucleoside–boron cluster conjugates in water and requires further derivatization of these molecules to improve their hydrophilicity. Therefore, it may be

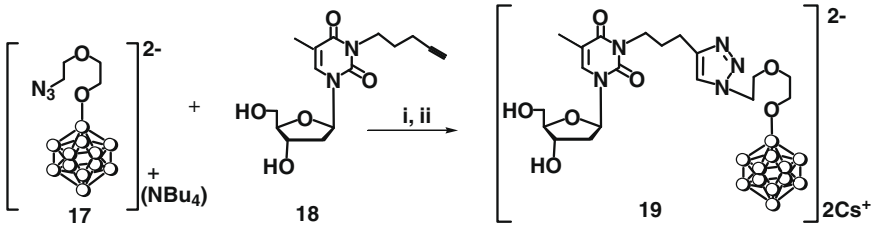


i. 7, 8 or 9, $CuSO_4 \cdot 5H_2O$/potassium ascorbate, *tert*-butanol/water (1:1)

Scheme 3. General method for the synthesis of nucleoside–boron cluster conjugates *via* click chemistry approach.



i. K_2CO_3, Bu_4NBr/CH_3CN; ii. CsF/CH_3OH; iii. Bu_4NF, H_2O, HCl/CH_2Cl_2; iv. CsF/CH_3OH



i. $CuSO_4 \cdot 5H_2O$/sodium ascorbate, CH_3CN; ii. CsF/CH_3OH

Scheme 4. Synthesis of nucleoside–dodecaborate conjugates.

surprising that in spite of high boron contents important for BNCT and good water solubility due to the presence of two negative charges dodecaborates ($B_{12}H_{12}^{2-}$) were not utilized so far for nucleoside modification. Recently, we reported the first synthesis of this type of novel nucleoside conjugate [45]. Two methods were proposed: (1) the nucleophilic cleavage of oxonium derivative of *closo*-dodecaborate by 3′,5′-bis (t-butyldimethylsilyl)-thymidine and (2) click reaction between B_{12}-based azide and 3*N*-(4-pentyn-1-yl)thymidine (*vide infra*). Both methods appeared as convenient approaches towards the synthesis of this new class of boron cluster-containing nucleoside derivatives. The target nucleosides **16** and **19** were obtained using methods described in Sections 3 and 5, with some modifications (Scheme 4). The yield of purified products was 70–80% [45].

6. Summary

Factors limiting applications of nucleoside–boron cluster conjugates include among others: (1) lack of variety among the boron clusters that are accessible to the medicinal chemists – by far most approaches utilized the *ortho*-carborane cage, (2) confinement of the nucleoside part of the conjugate to the pyrimidine nucleosides series – with very few exceptions only thymidine or uridine derivatives modified with boron unit were synthesized and studied till now, and (3) neglecting of the potential of metallacarboranes as modifying units for nucleosides.

Availability of new methodologies such as described above helps to bypass these limitations expanding the range of boron cluster bearing bioconjugates and make possible study of broad spectrum of pyrimidine as well as purine nucleoside derivatives in search for new biological activities and technical applications. Further approaches in particular directions are needed to facilitate exploration of a great potential of this fascinating class of bioorganic–inorganic conjugates.

Acknowledgments

This work was supported in part by the Polish Ministry of Science and Higher Education, Grant No. K152/H03/2007/09 and the Institutional grant from the Institute of Medical Biology PAS.

References

[1] H. Lodish, D. Baltimore, A. Berk, S.L. Zipursky, P. Matsudaira, J. Darnell, Molecular Cell Biology, W.H. Freeman and Co., New York, 1995.
[2] C.K. Chu (Ed.), Recent Advances in Nucleosides: Chemistry and Chemotherapy, Elsevier, Amsterdam, 2002.
[3] G.J. Peters (Ed.), Deoxy Nucleoside Analogs in Cancer Therapy Series: Cancer Drugs Discovery and Development, Humana Press Inc., Totowa, 2006.
[4] M.F. Hawthorne, Angew. Chem., Int. Ed. Engl. 32 (1993) 950.
[5] R.F. Barth, J.A. Coderre, M.G.H. Vicente, T.E. Blue, Clin. Cancer Res. 11 (2005) 3987.
[6] Z.J. Lesnikowski, J. Shi, R.F. Schinazi, J. Organomet. Chem. 581 (1999) 156.
[7] W. Tjarks, J. Organomet. Chem. 614 (2000) 37.
[8] A.S. Al-Madhoun, W. Tjarks, S. Eriksson, Mini-Rev. Med. Chem. 4 (2004) 341.
[9] Y. El-Kattan, N.M. Goudgaon, G. Fulcrand, D.C. Liotta, R.F. Schinazi, in: G.W. Kabalka (Ed.), Current Topics in the Chemistry of Boron, The Royal Society of Chemistry, Cambridge, 1988, p. 181.
[10] K. Imamura, Y. Yamamoto, Bioorg. Med. Chem. 6 (1996) 1855.
[11] G. Fulcrand-El Kattan, N.M. Goudgaon, N. Ilksoy, J.-T. Huang, K.A. Watanabe, J.-P. Sommadossi, R.F. Schinazi, J. Med. Chem. 37 (1994) 2583.
[12] J.-C.G. Graciet, J. Shi, R.F. Schinazi, Nucleosides Nucleotides 17 (1998) 711.
[13] S. Eriksson, B. Munch-Petersen, K. Johansson, H. Eklund, Cell. Mol. Life Sci. 59 (2002) 1327.
[14] Y. Yamamoto, T. Seko, H. Nakamura, Heteroatom Chem. 3 (1992) 239.
[15] A. Olejniczak, Proceeding of the ESF Exploratory Workshop "BioBor-Exploring New Opportunities of Boron Chemistry Towards Medicine", Lodz, Poland, May 9–12, 2008, p. 97.
[16] Z.J. Lesnikowski, Eur. J. Org. Chem. (2003) 4489.
[17] Z.J. Lesnikowski, Curr. Org. Chem. 11 (2007) 355.
[18] J.F. Valliant, K.J. Guenther, A.S. King, P. Morel, P. Schaffer, O.O. Sogbein, K.A. Stephenson, Coordin. Chem. Rev. 232 (2002) 173.
[19] K. Yamamoto, Y. Endo, Bioorg. Med. Chem. Lett. 11 (2001) 2389.
[20] Z.J. Lesnikowski, Collect. Czech. Chem. Commun. 72 (2007) 1646.
[21] A.B. Olejniczak, J. Plesek, O. Kriz, Z.J. Lesnikowski, Angew. Chem., Int. Ed. Engl. 42 (2003) 5740.
[22] F. Jelen, A.B. Olejniczak, A. Kourilova, Z.J. Lesnikowski, J. Palecek, Anal. Chem. 81 (2009) 840.
[23] A.B. Olejniczak, A. Sut, A.E. Wróblewski, Z.J. Lesnikowski, Vib. Spectrosc. 39 (2005) 177.
[24] Z.J. Leśnikowski, E. Paradowska, A.B. Olejniczak, M. Studzińska, P. Seekamp, U. Schüßler, D. Gabel, R.F. Schinazi, J. Plešek, Bioorg. Med. Chem. 13 (2005) 4168.
[25] B. Wojtczak, A. Semenyuk, A.B. Olejniczak, M. Kwiatkowski, Z.J. Lesnikowski, Tetrahedron Lett. 46 (2005) 3969.
[26] M. Matteucci, K.Y. Lin, S. Butcher, C. Moulds, J. Am. Chem. Soc. 111 (1991) 7767.
[27] D. Sawada, Y. Ito, Tetrahedron Lett. 42 (2001) 2501.
[28] J. Malmquist, S. Sjöberg, Tetrahedron 52 (1996) 9207.
[29] P. Cigler, M. Kozisek, P. Rezacova, J. Brynda, Z. Otwinowski, J. Pokorna, J. Plesek, B. Gruner, L. Doleckova-Maresova, M. Masa, J. Sedlacek, J. Bodem, H.G. Krausslich, V. Kral, J. Konvalinka, Proc. Natl. Acad. Sci. USA 102 (2005) 15394.
[30] I.H. Hall, C.B. Lackey, T.D. Kistler, R.W. Durham, J.M. Russell, R.N. Grimes, Anticancer Res. 20 (2000) 2345.
[31] V.I. Bregadze, I.B. Sivaev, S.A. Glazun, Anti-Cancer Agents Med. Chem. 6 (2006) 75.
[32] F. Teixidor, C. Viñas, A. Demonceau, R. Nuñez, Pure Appl. Chem. 75 (2003) 1305.
[33] A.B. Olejniczak, J. Plesek, Z.J. Lesnikowski, Chem. Eur. J. 13 (2007) 311.
[34] A.A. Semioshkin, I.B. Sivaev, V.I. Bregadze, Dalton Trans. (2008) 1.
[35] K.K. Oglivie, Can. J. Chem. 51 (1973) 3799.
[36] A.B. Olejniczak, P. Mucha, B. Grűner, Z.J. Lesnikowski, Organometallics 26 (2007) 3272.
[37] A.B. Olejniczak, M. Corsini, S. Fedi, P. Zanello, Z.J. Leśnikowski, Electrochem. Commun. 9 (2007) 1007.
[38] A.B. Olejniczak, B. Grűner, V. Šicha, S. Broniarek, ZJ. Lesnikowski, Electroanalysis (in press). doi: 10.1002/élan.200804434.
[39] H.C. Kolb, M.G. Finn, K.B. Sharpless, Angew. Chem., Int. Ed. Engl. 40 (2001) 2004.
[40] R. Huisgen, in: A. Padwa (Ed.), 1.3-Dipolar Cycloaddition Chemistry, Wiley, New York, 1984, pp. 1–176.
[41] B.A. Wojtczak, A. Andrysiak, B. Grűner, Z.J. Lesnikowski, Chem. Eur. J. 14 (2008) 10675.
[42] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, Angew. Chem., Int. Ed. 47 (2002) 2596.
[43] A. Olejniczak, B. Wojtczak, Z.J. Lesnikowski, Nucleosides Nucleotides Nucleic Acids 26 (2007) 1611.
[44] B. Wojtczak, A. Andrysiak, A. Olejniczak, Z.J. Leśnikowski, in: Proceedings of the Euroboron 4, Bremen, Germany, September 2–6, 2007, p. 85.
[45] A. Semioshkin, J. Laskova, B. Wojtczak, A. Andrysiak, I. Godovikov, V. Bregadze, Z.J. Lesnikowski, J. Organomet. Chem. 694 (2009) 1375.