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Synthesis of 3′-azido-3′-deoxythymidine (AZT)—*Cinchona* alkaloid conjugates via click chemistry: Toward novel fluorescent markers and cytostatic agents

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ABSTRACT

Novel nucleoside–*Cinchona* alkaloid conjugates were synthesized using 'click' chemistry approach based on the copper(I) catalyzed Huisgen azide–alkyne cycloaddition. Two series of conjugates were prepared employing 3′-azido-3′-deoxythymidine (AZT) as the azide component and the four 10,11-didehydro *Cinchona* alkaloids as well as their 9-*O*-propargyl ethers as the alkyne components. All obtained conjugates showed strong fluorescence emission and some of them exhibited marked cytotoxic activity in vitro.

Several 2′,3′-dideoxynucleosides, including 3′-azido-3′-deoxythymidine (AZT, zidovudine), have been used as antiretroviral drugs for the treatment of acquired immunodeficiency syndrome (AIDS).[1] In addition, there are some examples of application of AZT as an antitumor agent in the therapy of advanced colon cancer.[2] Wagner and co-workers also reported activity of AZT phosphoramidates against breast cancer.[3] On other hand, the major member of *Cinchona* alkaloids—quinine remained the antimalarial drug of choice until the 1940s and it is still used to treat chloroquine- and multidrug-resistant *Plasmodium falciparum* malaria.[4] Quinidine, a diastereoisomer of quinine, is widely used as an antiarrhythmic drug.[5] Apart from their pharmacological applications, quinine and quinidine display significant fluorescence emission owing to the presence of aromatic 6-methoxyquinoline ring and quinine sulfate is used routinely as a fluorescence quantum yield standard.[6]

In 2001 Sharpless and his co-workers introduced the 'click' chemistry concept and defined the criteria for 'click' reactions such as: wide in scope, high yield, modularity, ready available starting materials and reagents as well as simple reaction conditions.[7] Meldal[8] and Sharpless[9] independently established in 2002 that the Huisgen 1,3-dipolar cycloaddition reaction of organic azides and terminal alkynes could be efficiently catalyzed by copper(I) ions and thus performed at room temperature, resulting in the exclusive formation of 1,4-regioisomers of 1,2,3-triazole. This discovery had a tremendous impact on the development of conjugate synthesis of various organic compounds with azide and terminal alkyne functional groups. Thus, the copper(I) catalyzed azide–alkyne cycloaddition soon became the premier 'click' chemistry reaction.[10] 'Click' chemistry has also been recognized as valuable synthetic tool in the field of nucleosides, nucleotides, and DNA[11] since their azido and alkyne derivatives are readily accessible. However most of the published work focused on the preparation of nucleotide analogues or relatively simple nucleotide hybrids[12] and artificial linear or cyclic modified DNAs.[13] Less attention has been paid to the construction of functional hybrids consisting of nucleosides linked with other complex molecules such as natural products or fluorescence indicators which is crucial to the overall conjugate properties.[14] On other hand *Cinchona* alkaloids have been only rarely conjugated with other bioactive fragments[15] but to the best of our knowledge, nucleoside–*Cinchona* alkaloid hybrids linked with 1,2,3-triazole spacer have not been reported so far. It is worth adding that some successful attempts at the synthesis of 1,2,3-triazole modified nucleosides, using the Huisgen reaction, were reported prior to the advent of 'click' chemistry.[16]

The aim of our study was to synthesize novel nucleoside conjugates of *Cinchona* alkaloids with potential antiviral or anticancer as well as fluorescent properties. We expected that due to the presence of *Cinchona* alkaloid fluorophore its conjugates could potentially serve as reporter group for detection of DNAs.[17] Here, we report the synthesis of nucleoside–*Cinchona* alkaloid conjugates using 'click' chemistry approach based on the copper(I) catalyzed 1,3-dipolar Huisgen cycloaddition reaction. Two series of conjugates were prepared employing 3′-azido-3′-deoxythymidine (**1**) as the azide component and 10,11-didehydro *Cinchona* alkaloids **2a**–**d** as well as 9-*O*-propargyl *Cinchona* ethers **4a**–**d** as the alkyne components.

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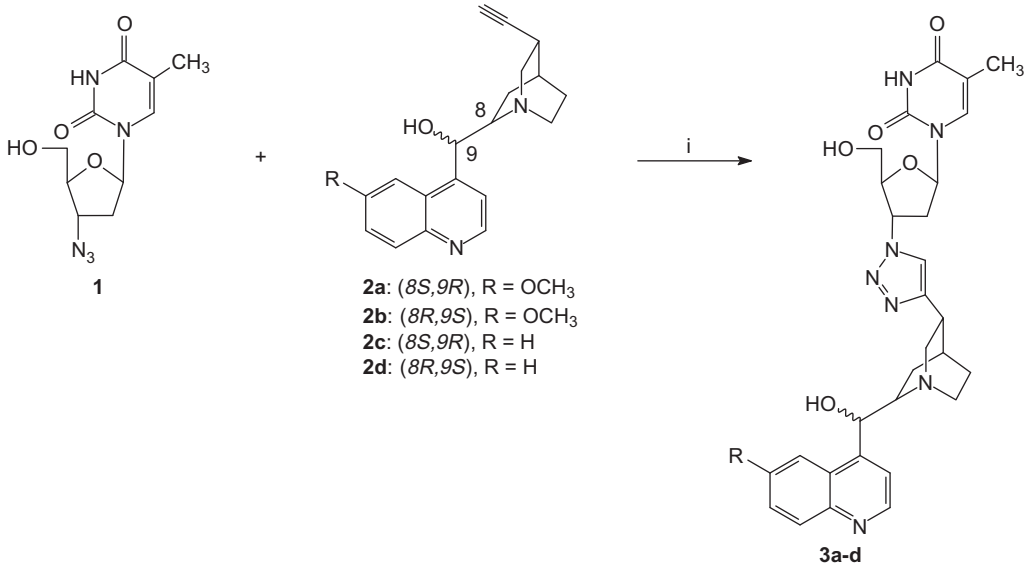


Scheme 1. Synthesis of 3′-azido-3′-deoxythymidine–10,11-didehydro *Cinchona* alkaloid conjugates **3a–d**. Reagents and conditions: (i) $CuSO_4$ (10 mol %), sodium ascorbate (40 mol %), THF/H_2O (3:1, v/v), rt, 12 h.

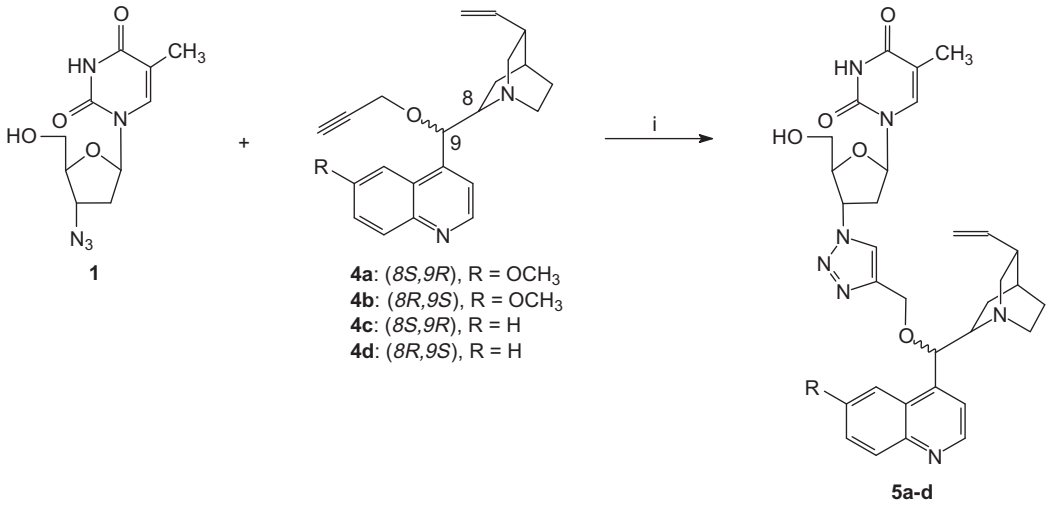
The first series of conjugates **3a–d** was synthesized by reaction of 3′-azido-3′-deoxythymidine (**1**)[18] with 10,11-didehydroquinine (**2a**)[19] and other 10,11-didehydro *Cinchona* alkaloids **2b–d** in the presence of copper(I) cations generated in situ from copper(II) sulfate and sodium ascorbate in THF–water (Scheme 1).[20]

The reaction afforded the corresponding conjugates **3a–d** as the single products in around 70% yield. It should be noted that the yield of conjugates **3a–d** is strongly dependent on the pH of the reaction mixture. Due to the high basicity of the modified *Cinchona* alkaloids a pH of the water–THF reaction mixture was around 8 and the yields of 70–75% were observed. Lowering the pH to 7 by addition of hydrochloric acid resulted in higher yields of 80–85%. A further decrease of the pH, however, led to reduction in the reaction yield.

We have also investigated the effect of organic co-solvent on the yield of the 'click' reaction. When *t*-butanol was used instead of THF, only a small decrease in the yield was observed. The reaction was also carried out using the Meldal procedure employing copper(I) iodide as a catalyst, and diisopropylethylamine (DIPEA) as a base in THF or acetonitrile but no expected improvement in the yield of **3a** was observed (60% yield). Although crude products **3a–d** could be precipitated from reaction mixture by addition of water it was more expedient to purify the products by silica gel column chromatography to remove traces of the starting materials and copper complexes.

The second series of conjugates **5a–d** was synthesized by the Huisgen cycloaddition of 3′-azido-3′-deoxythymidine (**1**) with 9-*O*-propargylquinine (**4a**)[21] and its congeners **4b–d** using similar procedure involving copper(I) cations generated in situ from copper(II) sulfate and sodium ascorbate in THF–water medium (Scheme 2).[20] This reaction also proceeded cleanly giving the corresponding products **5a–d** in 70–80% yield after column chromatography.

Fluorescence emission spectra (measured in acetonitrile) of starting 10,11-didehydro and 9-*O*-propargyl *Cinchona* alkaloids (compounds **2a–b** and **4a–b**, respectively) show strong emission band with the maximum centered at about 360 nm on excitation at 320 nm (Fig. 1). The fluorescence spectra of the respective



Scheme 2. Synthesis of 3′-azido-3′-deoxythymidine-9-*O*-propargyl *Cinchona* alkaloid conjugates **5a–d**. Reagents and conditions: (i) $CuSO_4$ (10 mol %), sodium ascorbate (40 mol %), THF/H_2O (3:1, v/v), rt, 12 h.

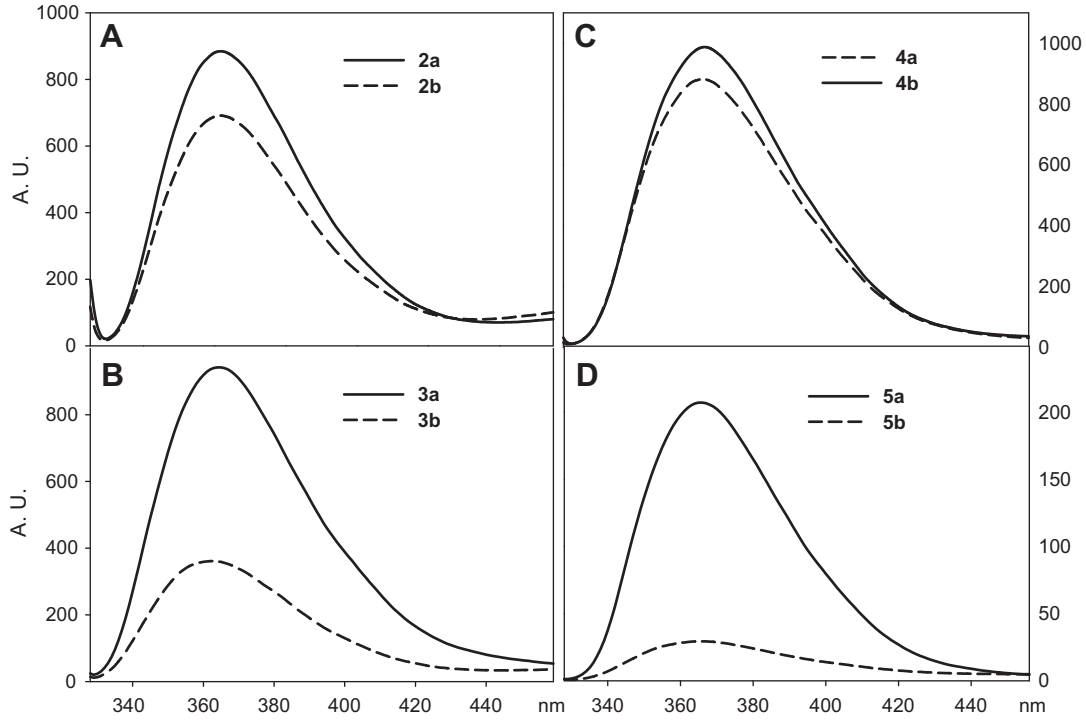


Figure 1. Fluorescence emission spectra of *Cinchona* alkynes **2a–b** and **4a–b** (upper panel, left and right column, respectively) and their conjugates **3a–b** and **5a–b** (bottom panel, left and right column, respectively) all spectra measured in acetonitrile ($c = 1 \times 10^{-5}$ mol/L) on excitation at 320 nm.

Table 1
Quantum yield of fluorescence of *Cinchona* alkynes **2a–b** and **4a–b** and their conjugates **3a–b** and **5a–b** calculated using 0.1 M quinine sulfate as standard ($\Phi = 0.53$)

Compound	Φ_T[a]
2a	2.34×10^{-2}
2b	1.74×10^{-2}
3a	4.334×10^{-2}
3b	2.997×10^{-2}
4a	3.96×10^{-2}
4b	2.77×10^{-2}
5a	7.198×10^{-2}
5b	7.784×10^{-3}

For other condition see Figure 1.
[a] Total quantum yield of fluorescence calculated from the whole spectrum.

Table 2
Cytotoxic activity of conjugates **3a–d** and **5a–d** against KB human cancer cells

Compound	ED_{50}[a] (μg/mL)
3a	1.3
3b	12
3c	11.6
3d	21
5a	2.1
5b	3.9
5c	>100
5d	>100
Cytosine arabinoside (standard)	0.99

[a] ED_{50} concentration of compound inhibiting by 50% protein biosynthesis in the cell line population.

nucleoside–*Cinchona* alkaloid conjugates **3a–b** and **5a–b** show similar characteristics both with respect to the emission wavelength and quantum yield (Table 1). For most conjugates a strong emission band with maximum centered at about 365 nm (Fig. 1) was observed indicating *Cinchona* 6-methoxyquinoline fluorophore presence. Somewhat unexpectedly, decrease in the intensity of the fluorescence emission was observed only for conjugate **5b** and it is likely to be due to the favoured spatial arrangement of the quinidine moiety and quenching effect of interacting nucleobase thymine.[22] These thymidine–*Cinchona* alkaloid conjugates **3a–b** and **5a–b** can be incorporated into DNA oligonucleotides at the 3′-end and their strong light emission make them promising fluorescent markers.

The in vitro cytotoxic activity of conjugates **3a–d** and **5a–d** was studied in human KB (*nasopharynx carcinoma*) tumor tissue culture using cytosine arabinoside (ED_{50} = 0.99 μg/mL) as a standard (Table 2).[23] In the first series of conjugates **3a–d** the most active was compound **3a** (ED_{50} = 1.3 μg/mL) the other compounds showed only a moderate activity (ED_{50} = 11.6–21 μg/mL). In the second series of conjugates **5a–d** the highest activity exhibited compounds **5a** (ED_{50} = 2.1 μg/mL), compound **5b** showed somewhat reduced activity (ED_{50} = 3.9 μg/mL) whereas **5c** and **5d** proved inactive. The findings show that despite different attachment site of *Cinchona* alkaloid pharmacophore to nucleoside moiety, there are some representative of both type conjugates (**3a** v. **5a** and **5b**) which exhibit considerable level of cytotoxicity.

In conclusion, two series of conjugates of 3′-azido-3′-deoxythymidine (**1**) and 10,11-didehydro *Cinchona* alkaloids **2a–d** as well as 9-*O*-propargyl *Cinchona* alkaloids **4a–d** were synthesized in good yields using the copper(I) catalyzed Huisgen azide–alkyne cycloaddition. Conjugates **3a–d** and **5a–d** showed significant fluorescence emission with potential applicability as fluorescent markers. In addition, conjugates **3a** and **5a–b** also exhibited promising cytotoxic activity in vitro.

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References and notes

1. De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115.
2. (a) Brunetti, I.; Falcone, A.; Calabresi, P.; Goulette, F. A.; Darnowski, J. W. *Cancer Res.* **1990**, *50*, 4026; (b) Darnowski, J. W.; Goulette, F. A. *Biochem. Pharmacol.* **1994**, *48*, 1797.
3. Iyer, V. V.; Griesgraber, G. W.; Radmer, M. R.; McIntee, E. J.; Wagner, C. R. *J. Med. Chem.* **2000**, *43*, 2266.
4. (a) Karle, J. M.; Karle, I. L.; Gerena, L.; Milhous, W. K. *Antimicrob. Agents Chemother.* **1992**, *36*, 1538; (b) Krishna, S.; White, N. J. *Clin. Pharmacokinet.* **1996**, *30*, 263.
5. Grace, A. A.; Camm, J. *N. Eng. J. Med.* **1998**, *338*, 35.
6. (a) Fletcher, A. N. *Photochem. Photobiol.* **1969**, *9*, 439; (b) Schulman, S. G.; Threatte, R. M.; Capomacchia, A. C.; Paul, W. L. *J. Pharm. Sci.* **1974**, *63*, 876.
7. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
8. Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
9. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
10. Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952.
11. Amblard, F.; Cho, J. H.; Schinazi, R. F. *Chem. Rev.* **2009**, *109*, 4207.
12. (a) Lakshman, M. K.; Singh, M. K.; Parrish, D.; Balachandran, R.; Day, B. W. *J. Org. Chem.* **2010**, *75*, 2461; (b) Lucas, R.; Zerrouki, R.; Granet, R.; Krausz, P.; Champavier, Y. *Tetrahedron* **2008**, *64*, 5467; (c) Lucas, R.; Neto, V.; Bouazza, A. H.; Zerrouki, R.; Granet, R.; Krausz, P.; Champavier, Y. *Tetrahedron Lett.* **2008**, *49*, 1004.
13. (a) Lietard, J.; Meyer, A.; Vasseur, J.-J.; Morvan, F. *J. Org. Chem.* **2008**, *73*, 191; (b) Isobe, H.; Fujino, T.; Yamazaki, N.; Guillot-Nieckowski, M.; Nakamura, E. *Org. Lett.* **2008**, *10*, 3729.
14. (a) Van Poecke, S.; Negri, A.; Gago, F.; Van Daele, I.; Solaroli, N.; Karlsson, A.; Balzarini, J.; Van Calenbergh, S. *J. Med. Chem.* **2010**, *53*, 2902; (b) Berndl, S.; Herzig, N.; Kele, P.; Lachmann, D.; Li, X.; Wolfbeis, O. S.; Wagenknecht, H.-A. *Bioconjugate Chem.* **2009**, *20*, 558; (c) Lolk, L.; Pøhlsgaard, J.; Jepsen, A. S.; Hansen, L. H.; Nielsen, H.; Steffansen, S. I.; Sparving, L.; Nielsen, A. B.; Vester, B.; Nielsen, P. *J. Med. Chem.* **2008**, *51*, 4957; (d) Seela, F.; Pujari, S. S. *Bioconjugate Chem.* ASAP **2010**.
15. (a) Walsh, J. J.; Coughlan, D.; Heneghan, N.; Gaynor, C.; Bell, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3599; (b) Sundermeier, U.; Dóbler, C.; Mehltretter, G. M.; Baumann, W.; Beller, M. *Chirality* **2003**, *15*, 127.
16. (a) Wigerinck, P.; Van Aerschot, A.; Claes, P.; Balzarini, J.; De Clercq, E.; Herdewijn, P. *J. Heterocycl. Chem.* **1989**, *26*, 1635; (b) Lazrek, H. B.; Taourirte, M.; Oulih, T.; Lebtoumi, M.; Barascut, J. L.; Imbach, J. L. *Nucleosides Nucleotides* **1997**, *16*, 1115; (c) Alvarez, R.; Velazquez, S.; San-Felix, A.; Aquaro, S.; De Clercq, E.; Perno, C.-F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185.
17. Saito, I.; Saito, Y.; Hanawa, K.; Hayashi, K.; Motegi, K.; Bag, S. S.; Dohno, C.; Ichiba, T.; Tainaka, K.; Okamoto, A. *Pure Appl. Chem.* **2006**, *78*, 2305.
18. Czernecki, S.; Valery, J.-M. *Synthesis* **1991**, 239.
19. Kacprzak, K. M.; Lindner, W.; Maier, N. M. *Chirality* **2008**, *20*, 441.
20. *General procedure for the synthesis of* ***3a–d*** *and* ***5a–d***: To a solution of 3′-azido-3′-deoxythymidine (70 mg, 0.262 mmol) and the appropriate 10,11-didehydro or 9-*O*-propargyl *Cinchona* alkaloid (compounds **2a–d** and **4a–d**, respectively, 0.262 mmol) in a 1:3 mixture of water and THF (4 mL) was added sodium ascorbate (21 mg, 0.105 mmol) followed by copper(II) sulfate pentahydrate (6.5 mg, 0.026 mmol, in 100 μL of water). The reaction mixture was stirred at room temperature for 12 h, at which time TLC indicated the reaction to be complete. Then the mixture was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel column using as an eluent the mixture chloroform–methanol (from 80:1 to 5:1, v/v) to afford products **3a–d** and **5a–d** (70–80% yield). Spectral data for selected compounds. Compound **3a**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21–1.27 (m, 1H, H-5*exo*), 1.47–1.60 (m, 1H, H-7*endo*), 1.60–1.73 (m, 2H, H-5*endo*, H-7*exo*), 1.81 (d, 3H, J = 1.0 Hz, 5-CH_3), 2.06 (br s, 1H, H-4), 2.55–2.73 (m, 4H, H-3, H-6*exo*, H-2′, H-2″ deoxyribose), 2.98–3.12 (m,1H, H-2*endo*), 3.25–3.41 (m, 2H, H-2*exo*, H-6*endo*), 3.54–3.71 (m, 3H, H-8, H-5′, H-5″ deoxyribose), 3.92 (s, 3H, O–CH_3), 4.12–4.18 (m, 1H, H-4′ deoxyribose), 5.25–5.32 (m, 1H, H-3′deoxyribose), 5.39 (d, 1H, J = 5.1 Hz, H-9), 6.39 (pseudo t, 1H, J = 6.6 Hz, H-1′ deoxyribose), 7.40 (dd, 1H, J = 9.2 and 2.6 Hz, H-7′), 7.52 (d, 1H, J = 4.5 Hz, H-3′), 7.55 (d, 1H, J = 2.6 Hz, H-5′), 7.82 (d, 1H, J = 1.0 Hz, H-6), 7.93 (d, 1H, J = 9.2 Hz, H-8′), 8.15 (s, 1H, triazole), 8.69 (d, 1H, J = 4.5 Hz, H-2′), 11.37 (br s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.26, 26.76, 27.38, 32.39, 37.08, 41.99, 50.07, 55.09, 55.60, 59.03, 60.15, 60.73, 70.22, 83.85, 84.48, 102.41, 109.62, 119.22, 121.11, 121.53, 126.90, 131.16, 136.26, 143.92, 147.47, 148.74, 150.01, 150.45, 156.93, 163.75. MS (ESI): m/z 591 ($M+H^+$), 613 ($M+Na^+$). Anal. Calcd for $C_{30}H_{35}N_7O_6$: C, 61.11; H, 5.98; N, 16.63. Found: C, 61.02; H, 5.93; N, 16.58. Compound **5a**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27–1.35 (m, 1H, H-5*exo*), 1.37–1.42 (m, 1H, H-7*endo*), 1.56–1.72 (m, 2H, H-5*endo*, H-7*exo*), 1.82 (s, 3H, 5-CH_3), 1.87 (br s, 1H, H-4), 2.62–2.76 (m, 4H, H-3, H-6*exo*, H-2′, H-2″ deoxyribose), 2.90 (m, 1H, H-2*endo*), 3.18–3.39 (m, 2H, H-2*exo*, H-6*endo*), 3.63–3.72 (m, 3H, H-8, H-6*endo*, H-5′, H-5″ deoxyribose), 4.01 (s, 3H, O–CH_3), 4.22 (m, 1H, H-4′ deoxyribose), 4.52 (s, 2H, O–CH_2), 4.96 (d, 1H, J = 10.4 Hz, H-11a), 5.04 (d, 1H, J = 17.1 Hz, H-11b), 5.35 (d, 1H, J = 5.1 Hz, H-9), 5.37–5.40 (m, 1H, H-3′deoxyribose), 5.83 (m, 1H, H-10), 6.44 (pseudo t, 1H, J = 6.6 Hz, H-1′ deoxyribose), 7.46 (dd, 1H, J = 9.2 and 2.3 Hz, H-7′), 7.58 (d, 1H, J = 3.8 Hz, H-3′), 7.67 (d, 1H, J = 2.3 Hz, H-5′), 7.86 (s, 1H, H-6), 7.99 (d, 1H, J = 9.2 Hz, H-8′), 8.38 (s, 1H, triazole), 8.78 (d, 1H, J = 3.8 Hz, H-2′), 11.39 (br s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.27, 26.88, 30.16, 31.20, 34.68, 37.20, 42.41, 56.55, 59.25, 60.36, 60.74, 61.75, 63.49, 69.78, 83.91, 84.51, 102.25, 109.65, 115.52, 118.83, 121.90, 124.14, 126.98, 131.29, 136.25, 139.20, 143.05, 144.08, 147.43, 149.61, 150.46, 157.67, 163.75. MS (ESI): m/z 631 ($M+H^+$), 653 ($M+Na^+$). Anal. Calcd for $C_{33}H_{39}N_7O_6$: C, 62.94; H, 6.24; N, 15.57. Found: C, 62.85; H, 6.17; N, 15.49.
21. Ishii, Y.; Fujimoto, R.; Mikami, M.; Murakami, S.; Miki, Y.; Furukawa, Y. *Org. Process Res. Dev.* **2007**, *11*, 609.
22. Seela, F.; Ming, X. *Helv. Chim. Acta* **2008**, *91*, 1181.
23. Hladon, B.; Goslinski, T.; Laskowska, H.; Baranowski, D.; Ostrowski, T.; Zeidler, J.; Ruszkowski, P.; Golankiewicz, B. *Pol. J. Pharmacol.* **2002**, *54*, 45.