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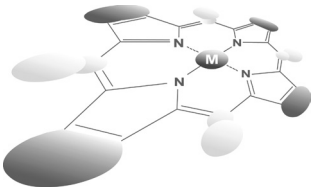

"Click chemistry" in the synthesis of the first glycoconjugates of bacteriochlorin series

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ABSTRACT: A regioselective synthesis of glycoconjugates based on bacteriochlorophyll *a* and lactose derivatives has been carried out. The conjugation was achieved *via* 1,3-dipolar cycloaddition of bacteriochlorins containing a terminal triple bond and a lactose azide derivative. The conjugates obtained in this way had one or two disaccharide fragments attached to pyrrol A, the exocyclic imide ring of the tetrapyrrolyc macrocycle, or to both positions. Exhaustive NMR analysis by 1D and 2D NMR experiments (^{1}H-^{1}H COSY, TOCSY, ROESY, ^{1}H-^{13}C HSQC, HMBC, and ^{1}H-^{15}N HMBC) allowed us to determine the structures and configurations of the glycoconjugates obtained. A bioassay of the glycoconjugates using the Hep2 cell line showed that the highest efficiency was observed for the glycosylated bacteriopurpurinimide containing a lactose residue at pyrrole ring A.

KEYWORDS: bacteriochlorin, bacteriopurpurinimide, glycoconjugates, click chemistry, triazole, photodynamic therapy of cancer, photosensitizers, lactose, galectin.

INTRODUCTION

Photodynamic therapy (PDT) of cancer is an efficient noninvasive method for the treatment of malignant tumors involving three low-toxic components, namely oxygen, laser radiation and a photosensitizer (PS), which enter the cancer cell and induce a cytotoxic effect [1–3]. Pigments with an absorption maximum in the near-IR spectral region where the tissue has the highest permeability to light are used as the photosensitisers [4]. These requirements are satisfied by bacteriochlorophyll *a* derivatives that absorb in the 800 nm region and have low dark toxicity [5]. The high selectivity of PS accumulation in the tumor required to increase PDT efficiency may be achieved by binding the pigment molecules with various systems of targeted delivery. Many types of tumors are known to manifest an enhanced expression of galectins, *i.e.* proteins with high affinity to β-galactosides [6]. In view of this, addition of galactose or lactose to the tetrapyrrole macrocycle ensures that conjugates are specifically bound to tumor cells and thus enhances the method efficiency [7, 8]. Furthermore, addition of carbohydrate units to the hydrophobic tetrapyrrole macrocycle allows the pigment solubility in aqueous solutions to be improved and can be used to make the molecule amphiphilic, thus affecting the accumulation and localization of a PS in a tumor cell [9–13]. The synthesis of carbohydrate — porphyrin conjugates attracts the attention of many scientific groups in the world, but studies

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dealing with glycochlorins and glycobacteriochlorins are rather scarce [14–16]. This is primarily due to the smaller synthetic availability of chlorins and bacteriochlorins in comparison with porphyrins. However, owing to their excellent spectral characteristics, photosensitizers based on di- and tetrahydroporphyrins are the most promising candidates for creating efficient second-generation drugs for the PDT of cancer.

In our previous papers, we reported the synthesis of the bacteriochlorin *p* conjugate with lactose attached to additional exocycle E [17, 18].

In continuation of these studies, we used the well-known 1,3-dipolar cycloaddition [19] for regioselective incorporation of a sugar residue into the imide exocycle, to pyrrole ring A, or to both positions at once. In this study, we used *in vitro* experiments with the Hep2 cell line in an attempt to find the optimum position of the lactose residue in the macrocycle to maximize the glycoconjugate biological efficiency.

EXPERIMENTAL

General

The solvents were purified and prepared using standard procedures. All reactions were carried out under argon in air-free solvents and with protection from direct light. Bacteriopurpurin methyl ester (**1**) [20] and *N*-dimethylaminobacteriopurpurinimide methyl ester (**7**) [21] were obtained using a known procedure. Electronic spectra were recorded using a Jasco-UV 7800 spectrophotometer. NMR spectra were recorded at 25.7 °C on Bruker DPX 300 and Bruker Avance 600 spectrometers. The signals of carbon atoms and those of residual protons in deuterated solvents were used to calibrate the ^{13}C and ^{1}H scales, respectively [22]. ^{15}N NMR spectra were recorded using 100% nitromethane as the external standard (δN = 0.0). All experiments were based on standard Bruker techniques. TOCSY spectra were recorded with 60 ms MLEV-17 spin-lock duration; ROESY spectra were recorded with 150 ms spin-lock duration. ^{1}H-^{13}C and ^{1}H-^{15}N gHMBC spectra were recorded with 60 and 50 ms delay for evolution of long-range couplings, respectively. Mass spectra were obtained on a Bruker Ultraflex TOF/TOF time-of-flight mass spectrometer using the MALDI method, with 2,5-dihydroxybenzoic acid (DHB) as the matrix. IR spectra were obtained on a Bruker EQUINOX 55 (KBr) instrument. Column chromatography was carried out on 40/60 silica gel (Merck). Preparative TLC was performed on silica gel 60 (Merck) using 20 × 20 cm plates with a layer thickness of 1 mm. Analytical TLC was carried out on Kieselgel 60 F245 plates (Merck).

Synthesis

***N*-propargylbacteriopurpurinimide methyl ester (2).** Propargylamine (100 μl, 1.56 mmol) was added to a solution of bacteriopurpurin methyl ester **1** (100 mg, 0.17 mmol) in chloroform (5 mL) and the mixture was refluxed under a positive argon pressure. After 16 h, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified by preparative thin-layer chromatography in the dichloromethane/methanol system (49:1). The yield was 65 mg (61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H, ppm 9.22 (H, s, 5-H), 8.80 (H, s, 10-H), 8.62 (H, s, 20-H), 5.30 (H, m, 17-H), 5.27, 5.22 (2H, AB part of ABX, J_{AB} = 16.5 Hz, $J_{AX} = J_{BX}$ = 2.4 Hz, N–CH_2), 4.29 (2H, m, 7-H, 18-H), 4.092 (H, m, 8-H), 3.70 (3H, s, 12-CH_3), 3.58 (3H, s, 17^4-CH_3), 3.53 (3H, s, 2-CH_3), 3.17 (3H, s, 3^2-CH_3), 2.72 (H, m, 17^{2a}-CH_2), 2.37 (3H, m, 8^{1a}-CH_2, 17^{1a}-CH_2, 17^{2b}-CH_2), 2.30 (H, part X of ABX, *J* = 2.4 Hz, C≡CH), 2.01 (2H, m, 8^{1b}-CH_2, 17^{1b}-CH_2), 1.81 (3H, d, *J* = 7.24 Hz, 7^1-CH_3), 1.71 (3H, d, *J* = 7.20 Hz, 18^1-CH_3), 1.11 (3H, t, *J* = 7.38 Hz, 8^2-CH_3), -0.42 (H, NH), -0.66 (H, NH). MS (MALDI): *m/z* 633.5 $[M]^+$, 598.5 $[M - CH_2CCH]^+$. UV-vis ($CHCl_3$): λ, nm (ε, $M^{-1}.cm^{-1}$) 365 (84700), 415 (37300), 547 (21500), 826 (57000). IR (KBr): ν, cm^{-1} 3290 (C–H alkyne), 2110 (C–C alkyne).

3-deacetyl-3-(α-(propargylamino)ethyl)-*N*-dimethylaminobacteriopurpurinimide methyl ester (8). Propargylamine (400 μl, 6.12 mmol) was added to a solution of *N*-dimethylaminobacteriopurpurinimide methyl ester **7** (120 mg, 0.19 mmol) in chloroform (5 mL) and the mixture was refluxed under a positive argon pressure. After 30 h, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. The residue was chromatographed on silica gel in the chloroform/methanol system (49:1). The yield of the corresponding Schiff base amounted to 83 mg (65%). After that, sodium borohydride (9 mg, 0.25 mmol) was added to a solution of the resulting Schiff base (83 mg, 0.12 mmol) in anhydrous methanol (2 mL) and the mixture was stirred for 15 min at room temperature. The reaction mixture was transferred into a separating funnel and diluted with dichloromethane (30 mL) and water (100 mL); 1 N HCl (2 mL) was added and the system was extracted with dichloromethane (3 × 30 mL). The combined extracts were washed with water, dried with anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash chromatography, using the 5% methanol/dichloromethane system as the eluent. The yield was 71 mg (86%) as a mixture of two diastereomers. ^{1}H NMR (600 MHz, $CDCl_3$): δ_H, ppm 9.09 and 8.98 (H, each s, 5-H), 8.59 and 8.58 (H, each s, 10-H), 8.32 and 8.30 (H, each s, 20-H), 5.26 and 5.20 (2H, AB part of ABX, J_{AB} = 16.7 Hz, $J_{AX} = J_{BX}$ = 2.4 Hz, NH-$\underline{CH_2}$), 5.24 (H, m, 17-H), 4.23 (H, m, 7-H), 4.19 (2H, m, 18-H, 3^1-H), 3.98 (H, m, 8-H), 3.68 (H, m, $\underline{NH}$-CH_2), 3.62 (3H, s, 12^1-CH_3), 3.61 (3H, s, 17^4-CH_3), 3.58 (6H, s, $N(CH_3)_2$), 3.30 and 3.28 (3H, each s, 2^1-CH_3), 2.68 (H, m, 17^{2a}-CH_2), 2.36 (3H, m, 17^{1a}-H, 17^{2b}-H, 8^{1a}-H), 2.28 (H, part X of ABX, *J* = 2.4 Hz, C≡CH), 2.04 (H, m, 8^{1b}-H), 1.99 (H, m, 17^{1b}-H), 1.81 (3H, d, *J* = 7.4 Hz, 7^1-CH_3), 1.78 (3H, d, *J* = 7.3 Hz, 18^1-CH_3), 1.67 (3H, d,

J = 7.4 Hz, 3^2-CH_3), 1.14 and 1.13 (3H, each t, J = 7.3 Hz, 8^2-CH_3), 0.14 and 0.10 (H, each s, NH), -0.22 and -0.25 (H, each s, NH). MS (MALDI): *m/z* 678.4 $[M]^+$, 634.4 $[M–N(CH_3)_2]^+$, 623.4 $[M - NHCH_2CCH]^+$. UV-vis ($CHCl_3$): λ, nm (ε, $M^{-1}.cm^{-1}$) 367 (89000), 415 (50300), 537 (31600), 803 (43400). IR (KBr): ν, cm^{-1} 3292 (C–H alkyne), 2124 (C–C alkyne).

3-deacetyl-3-(α-(propargylamino)ethyl)-*N*-propargylbacteriopurpurinimide methyl ester (11). Propargylamine (100 μl, 6.12 mmol) was added to a solution of bacteriopurpurin methyl ester **1** (100 mg, 0.17 mmol) in chloroform (5 mL) and the mixture was refluxed under a positive argon pressure. After 40 h, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. The residue was chromatographed on silica gel in the chloroform/methanol system (49:1). The yield of the corresponding Schiff base amounted to 85 mg (75%). After that, sodium borohydride (9 mg, 0.25 mmol) was added to a solution of the resulting Schiff base (85 mg, 0.13 mmol) in anhydrous methanol (2 mL) and the mixture was stirred for 15 min at room temperature. The reaction mixture was transferred into a separating funnel and diluted with dichloromethane (30 mL) and water (100 mmL); 1 N HCl (2 mL) was added and the system was extracted with dichloromethane (3 × 30 mL). The combined extracts were washed with water, dried with anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash chromatography, using the 5% methanol/dichloromethane system as the eluent. The yield was 76 mg (90%) as a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H, ppm 9.09 and 8.95 (H, each s, 5-H), 8.56 and 8.55 (H, each s, 10-H), 8.28 and 8.27 (H, each s, 20-H), 5.47 and 5.42 (H, m, 17-H), 5.26 and 5.20 (4H, AB part of ABX, J_{AB} = 16.5 Hz, $J_{AX} = J_{BX}$ = 2.4 Hz, N-CH_2), 4.20 (H, m, 7-H), 4.15 (2H, m, 18-H, 3^1-H), 3.96 (H, m, 8-H), 3.61 (3H, s, 12-CH_3), 3.58 (3H, s, 17^4-CH_3), 3.28 and 3.25 (3H, each s, 2^1-CH_3), 2.67 (H, m, 17^{2a}-CH_2), 2.35 (3H, m, 8^{1a}-CH_2, 17^{1a}-CH_2, 17^{2b}-CH_2), 2.28 (2H, part X of ABX, J = 2.4 Hz, C≡CH), 2.03 (H, m, 8^{1b}-CH_2), 1.98 (H, m, 17^{1b}-H), 1.93 and 1.92 (3H, each d, J = 7.4 Hz, 7^1-CH_3), 1.79 and 1.77 (3H, each d, J = 7.20 Hz, 18^1-CH_3), 1.67 (3H, s, 3^2-CH_3), 1.14 and 1.13 (3H, each t, J = 7.3 Hz, 8^2-CH_3), 0.31 and 0.29 (H, each s, NH), -0.08 and -0.10 (H, each s, NH). MS (MALDI): *m/z* 672.5 $[M]^+$, 618.5 $[M–NHCH_2CCH]^+$. UV-vis ($CHCl_3$): λ, nm (ε, $M^{-1}.cm^{-1}$) 367.5 (94000), 418.5 (40100), 539.5 (37200), 788.5 (39500). IR (KBr): ν, cm^{-1} 3293 (C–H alkyne), 2123 (C–C alkyne).

***N*-(*N*-azidoacetylglycyl)-4-O-(β-D-galacto-pyranosyl)-β-D-glucopyranosylamine (4).** DMF (5.6 mL) was added to a solution of 4-O-(β-D-galactopyranosyl)-*N*-glycin-β-D-glucopyranosylamine (0.4 g, 1 mmol) in H_2O (1.4 mL), the solution was cooled with ice and succinimide azidoacetate (0.3 g, 1.5 mmol) was added with stirring. The solution was kept for 2 h at 20 °C and for 16 h at 5 °C. The reaction mixture was filtered. The filtrate was concentrated *in vacuo* at 10 Torr, then at 1 Torr to a volume of 3 mL, diluted with Et_2O (30 mL) with stirring, and kept for 16 h at -18 °C. The precipitate was filtered off, washed with Et_2O (30 mL), acetone (30 mL), and an acetone — MeOH mixture (1:1) (30 mL) and then dried. The residue was suspended in MeOH (10 mL); the precipitate was filtered off, washed with MeOH and Et_2O and dried to give 0.4 g (83%) of an amorphous compound, $[\alpha]_D^{20}$ +2.8 (*c* 1, H_2O). Found (%): C, 39.75; H, 5.91; N, 14.10. $C_{16}H_{27}N_5O_{12}$. Calcd. (%): C, 39.92; H, 5.65; N, 14.55. ^{1}H NMR (300 MHz, D_2O): δ_H, ppm 3.40–3.60 (m, 2 H); 3.61–3.81 (m, 10 H); 3.82–3.98 (m, 2 H); 4.04 (br. s, 2 H, C$\underline{H}_2$N); 4.11 (br. s, 2 H, C$\underline{H}_2$N); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

General method for the synthesis of glycoconjugates (5,9,12). A solution of the corresponding propargyl derivative of bacteriochlorin *p* (0.06 mmol, 1 equiv.), and lactose peracetate azide derivative **4** (0.08 mmol, 1.3 equiv.) (per one C≡CH group), diisopropylethylamine (1.00 mmol), and copper(I) iodide (0.006 mmol) (per one C≡CH group) in dichloromethane (6 mL) was stirred for 30 min at room temperature. The reaction mixture was concentrated *in vacuo* and separated by preparative TLC using dichloromethane/methanol (19:1) as the eluent. **Glycoconjugate (5).** Yield 80%. NMR data are presented in Table 1. MS (MALDI): *m/z* 1409.4 $[M]^+$, 1431.4 $[M + Na]^+$, 1447.4 $[M + K]^+$. UV-vis ($CHCl_3$): λ, nm (ε, $M^{-1}.cm^{-1}$) 364 (83000), 416 (44200), 548 (33000), 828 (60500). **Glycoconjugate (9).** Yield 83%. NMR data are presented in Table 1. MS (MALDI): *m/z* 1452.5 $[M]^+$, 1409.4 $[M – N(CH_3)_2]^+$, 831.1 $[M–Bhl]^+$, 623.1 $[M–Lac]^+$. UV-vis ($CHCl_3$): λ, nm (ε, $M^{-1}.cm^{-1}$) 366 (74600), 412 (41800), 534.5 (29100), 805 (56500). **Glycoconjugate (12).** Yield 70%. NMR data are presented in Table 2. MS (MALDI): *m/z* 2222.7 $[M]^+$, 1393.4 $[M–Lac]^+$, 831.1 $[M–Bhl(Lac)]^+$. UV-vis ($CHCl_3$): λ, nm (ε, $M^{-1}.cm^{-1}$) 367 (84000), 415 (49500), 536 (33000), 806 (54500).

General method for the deacylation of glycoconjugates (6,10,13). A 1 M solution of sodium methoxide (100 μl) in anhydrous methanol was added to a solution of the corresponding glycoconjugate (0.02 mmol) in a 1:1 mixture of dichloromethane and anhydrous methanol (2 mL) and the mixture is stirred for 1 h. The reaction mixture is then neutralized with glacial acetic acid (30 μL), diluted with dichloromethane (10 mL), washed with a $NaHCO_3$ solution, and extracted with chloroform (5 × 10 mL). The combined extracts are dried with sodium sulfate, concentrated *in vacuo*, and chromatographed on a plate using dichloromethane/methanol system (2:1) as the eluent. **Glycoconjugate (6).** Yield 66%. MS (MALDI): *m/z* 1114.2 $[M]^+$, 1137.2 $[M + Na]^+$. UV-vis (H_2O/1% Cremophor): λ, nm (ε, $M^{-1}.cm^{-1}$) 365 (88000), 415 (45000), 550 (33000), 830 (61000). **Glycoconjugate (10).** Yield 70%. MS (MALDI): *m/z* 1158.5 $[M]^+$, 1181.2 $[M + Na]^+$, 1197.5 $[M + K]^+$, 1115.4 $[M–N(CH_3)_2]^+$. UV-vis (H_2O/1% Cremophor): λ, nm

Table 1. ^{1}H, ^{13}C and ^{15}N NMR data for glycoconjugates **5** and **9**

Bacteriochlorin ρ ("**Bchl**")					
^{13}C or (^{15}**N**)	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	5	**9**		**5**	**9**
NH(A)	(**-248.9**)	**(-251.7)**	NH(A)	-0.67, **s**	-0.28, **s**
N(B)	(**-73.8**)	**(-76.6)**			
NH(C)	(**-254.4**)	**(-249.8)**	NH(C)	-0.46, **s**	0.06, **s**
N(D)	(**-87.1**)	**(-91.3)**			
C-1	137.2	141.1			
C-2	134.3	132.9			
C-2^1 (-CH_3)	13.5	11.1	H-2^1	3.53, **s**	3.27, **s**
C-3	132.2	137.9 137.9*			
C-3^1	198.4	51.6 51.2*	H-3^1		5.31, **m** 5.33*, **m**
C-3^2 (-CH_3)	33.1	23.6 23.6*	H-3^2	3.16, **s**	1.99, **d**, *7.4* 2.02*, **d**, *7.4*
N-3^2		**(-326.9)** **(-326.2)**			
C-4	135.8	132.7			-
C-5 (-CH=)	101.9	99.1 99.0*	H-5	9.18, **s**	9.04, **s** 8.94*, **s**
C-6	170.1	170.4 170.4*			
C-7	47.5	48.2 48.1*	H-7	4.26, **m**	4.20, **m** 4.22*, **m**
C-7^1 (-CH_3)	23.1	22.8 22.9*	H-7^1	1.77, **d**, *7.4*	1.79, **d**, *7.4* 1.77*, **d**, *7.4*
C-8	55.2	55.2 55.2*	H-8	4.02, **m**	4.00, **m** 3.98*, **m**
C-8^1 (-***C***H_2-CH_3)	29.9	30.2	H-8^{1a} H-8^{1b}	2.31, **m** 2.00, **m**	2.33, **m** 2.04, **m**
C-8^2 (-CH_2-***C***H_3)	10.7	10.7	H-8^2	1.07, **t**, *7.3*	1.12, **t**, *7.3*
C-9	164.2	161.0			
C-10 (-CH=)	102.0	101.3 101.3*	H-10	8.71, **s**	8.60, **s**
C-11	134.2	129.8			
C-12	133.8	131.5			
C-12^1 (-CH_3)	12.1	11.8	H-12^1	3.62, **s**	3.62, **s**
C-13	115.5	114.1			
C-13^1 (>C=O)	162.9	163.5			
N-13^2	(**-208.5**)	**(-176.2)**			
N-13^3		**(-320.3)**			
C-13^4 (-$N(CH_3)_2$)		44.4	H-13^4 (-$N(CH_3)_2$)		3.35, **s** 3.34, **s**

(*Continued*)

Table 1. (*Continued*)

Bacteriochlorin ρ ("**Bchl**")					
^{13}C or (^{15}**N**)	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	5	**9**		**5**	**9**
C-14	135.2	134.6			
C-15	134.0	133.5			
C-15^{1} (>C=O)	167.2	167.3			
C-16	173.0	172.6			
C-17	54.3	54.0 53.9*	H-17	5.29, **m**	5.19, **m**
C-17^{1} (-***C***H$_2$-CH$_2$-CO$_2$CH$_3$)	31.5	31.2	H-17^{1a} H-17^{1b}	2.33, **m** 1.90, **m**	2.38, **m** 1.94, **m**
C-17^{2} (-CH$_2$-***C***H$_2$-CO$_2$CH$_3$)	32.3	32.3	H-17^{2}	2.68, **m** 2.37, **m**	2.75, **m** 2.43, **m**
C-17^{3} (-CH$_2$-CH$_2$-***C***O$_2$CH$_3$)	174.0	174.0			
C-17^{4} (-CH$_2$-CH$_2$-CO$_2$***C***H$_3$)	51.6	51.4	H-17^{4}	3.57, **s**	3.57, **s**
C-18	48.6	49.3	H-18	4.24, **m**	4.20, **m**
C-18^{1} (-CH$_3$)	24.0	23.4 23.5*	H-18^{1}	1.72, **d**, *7.3*	1.69, **d**, *7.3* 1.67*, **d**, *7.3*
C-19	170.3	173.9			
C-20 (-CH=)	97.0	94.8 94.8*	H-20	8.61, **s**	8.33, **s**
Triazole and spacer ("**Spr**")					
^{13}C or (^{15}**N**)	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	5	**9**		**5**	**9**
C-1 (>N-***C***H$_2$-C<)	35.0	42.9 42.8*	H-1^{a} H-1^{b}	5.89, **d**, *15.0* 5.64, **d**, *15.0*	4.06, 4.04*, **d**, *15.0* 4.02, 4.01*, **d**, *15.0*
C-2	145.7	146.4			
C-3 (-CH=)	125.6	123.7 123.8*	H-3	8.11, **s**	7.61, **s** 7.58*, **s**
N-4	(**-144.7**)	**(-145.8)**			
N-5	(**-22.3**)	**(-19.8)**			
N-6	(**-27.6**)	**(-28.5)**			
C-7	52.8	55.2 55.2*	H-7^{a} H-7^{b}	5.22, **d**, *16.9* 5.17, **d**, *16.9*	5.12, **d**, *16.9* 5.08, **d**, *16.9*
C-8 (>C=O)	166.6	166.0			
N-9	(**-276.9**)	**(-277.7)**	NH-9	7.13, **t**, *5.5*	7.03, **t**, *5.5*
C-10	43.5	43.2	H-10^{a} H-10^{b}	3.90, **dd**, *16.5*, *5.5* 3.86, **dd**, *16.5*, *5.5*	3.92, **dd**, *16.5*, *5.5* 3.88, **dd**, *16.5*, *5.5*
C-11 (>C=O)	168.8	168.6	H-11		
N-12	(**-261.3**)	**(-261.6)**	NH-12	7.07, **d**, *9.2*	6.96, **d**, *9.2* 6.98*, **d**, *9.2*

(*Continued*)

Table 1. (*Continued*)

β-D-galactopyranose (**"Gal"**)					
^{13}C	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	5	**9**		**5**	**9**
C-1	100.8	100.8	H-1	4.47, **d**, *8.1*	4.47, **d**, *8.1*
C-2	69.1	69.0	H-2	5.08, **dd**, *10.3*, *8.1*	5.09, **dd**, *10.3*, *8.1*
C-3	71.0	70.9	H-3	4.95, **dd**, *10.3*, *3.3*	4.95, **dd**, *10.3*, *3.3*
C-4	66.7	66.6	H-4	5.34, **d**, *3.3*	5.36, **d**, *3.3*
C-5	70.7	70.7	H-5	3.87, **t**, *7.0*	3.87, **t**, *7.0*
C-6	60.9	60.7	H-6[a] H-6[b]	4.12, **m** 4.07, **m**	4.14, **m** 4.06, **m**
OAc (-***C***O-)	168.9–170.3	168.9–170.2			
OAc (-***C***H$_3$)	20.4–20.7	20.5–20.7	OAc	1.98–2.09, **s**	1.98–2.09, **s**
β-D-glucopyranose (**"Glc"**)					
^{13}C	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	5	**9**		**5**	**9**
C-1	78.1	78.1	H-1	5.13, **t**, *9.2*	5.16, **t**, *9.2* 5.14*, **t**, *9.2*
C-2	70.8	70.8	H-2	4.84, **t**, *9.2*	4.82, **t**, *9.5*
C-3	72.2	72.2	H-3	5.27, **t**, *9.5*	5.29, **t**, *9.5*
C-4	75.9	75.7	H-4	3.77, **t**, *9.5*	3.79, **t**, *9.5*
C-5	74.5	74.6	H-5	3.72, **m**	3.76, **m**
C-6	61.9	61.7	H-6[a] H-6[b]	4.41, **m** 4.11, **m**	4.45, **m** 4.06, **m**
OAc (-***C***O-)	170.3–171.7	170.2–171.6			
OAc (-***C***H$_3$)	20.6–20.7	20.4–20.7	OAc	1.99–2.07, **s**	1.97–2.09, **s**

* Signals of two diastereomers.

(ε, $M^{-1}.cm^{-1}$) 370 (76000), 412 (42000), 535 (30000), 805 (57000). **Glycoconjugate (13).** Yield 55%. MS (MALDI): *m/z* 1634.7 $[M]^+$, 1657.6 $[M + Na]^+$, 1110.4 $[M–Lac]^+$. UV-vis (H_2O/1% Cremophor): λ, nm (ε, $M^{-1}.cm^{-1}$) 366 (84000), 415 (50500), 535 (33000), 805 (56000).

Analysis of absorption and fluorescence spectra of compounds 6, 7, 10, 13

During the studies, we assessed changes in the position of the spectral maximum, optical absorption and fluorescence values, and changes in the spectrum profile in time.

The solutions for the study were prepared ex tempore; the required concentrations were achieved by sequential dilutions of the initial solution. The initial solution concentration was 1 mg/mL. Eagle's minimum essential medium with 7% fetal calf serum was used as the solvent.

The concentration of photosensitizers in the solution was 20 μg/mL in experiments on assessment of absorption spectra; in fluorescent analyses, it was 5 μg/mL conjugate (**10**) and 20 μg/mL for purpurinimide (**7**), conjugates (**6** and **13**). Absorption spectra were recorded on a Genesys 2 spectrophotometer in the wavelength range from 500 to 800 nm; fluorescence was excited with a He–Ne laser (generation wavelength 632.8 nm; spectral range 400 to 800 nm), optical resolution 2 nm. Measurements were carried out by a contact method using a Lesa laser spectral analyzer ("BioSpek", Russia). Spectra were recorded immediately after the preparation of solutions and after two hours of incubation at room temperature.

In vitro studies

Experiments were carried out using the human laryngeal cancer cell line Hep-2. The human laryngeal carcinoma HEp-2 cells were grown (37 °C, 5% CO_2) in Eagle's minimum essential medium with phenol red

Table 2. ^{1}H and ^{13}C NMR data for glycoconjugate **12**

Bacteriochlorin ρ ("**Bchl**")			
^{13}C	δ, ppm	^{1}H	δ, ppm, multiplicity, *J*, Hz
		NH(A)	0.01, **s**
		NH(C)	0.40, **s**
C-1	**		
C-2	**		
C-2^1 (-CH_3)	11.6	H-2^1	3.42, **s**
C-3	**		
C-3^1	51.5 51.7*	H-3^1	5.26, **m** 5.24*, **m**
C-3^2 (-CH_3)	24.1 23.9*	H-3^2	1.87, **d**, *7.4* 1.89*, **d**, *7.4*
C-4	**		
C-5 (-CH=)	100.4 100.7*	H-5	9.15, **s** 9.25*, **s**
C-6	**		
C-7	49.0	H-7	4.12, **m**
C-7^1 (-CH_3)	22.9	H-7^1	1.77 **d**, *7.4*
C-8	55.5	H-8	3.85, **m**
C-8^1 (-***C***H_2-CH_3)	30.6	H-8^{1a} H-8^{1b}	2.25, **m** 1.91, **m**
C-8^2 (-CH_2-***C***H_3)	10.9	H-8^2	1.01, **t**, *7.3*
C-9	**		
C-10 (-CH=)	101.9	H-10	8.52, **s**
C-11	**		
C-12	**		
C-12^1 (-CH_3)	11.1	H-12^1	3.24, **s**
C-13	**		
C-13^1 (>C=O)	**		
C-14	**		
C-15	**		
C-15^1 (>C=O)	**		
C-16	**		
C-17	54.5	H-17	5.13, **m**
C-17^1(-***C***H_2-CH_2-CO_2CH_3)	32.2	H-17^{1a} H-17^{1b}	2.28, **m** 1.84, **m**
C-17^2(-CH_2-***C***H_2-CO_2CH_3)	32.7	H-17^2	2.58, **m** 2.36, **m**
C-17^3(-CH_2-CH_2-***C***O_2CH_3)	173.8		
C-17^4(-CH_2-CH_2-CO_2***C***H_3)	51.6	H-17^4	3.51, **s**
C-18	50.0	H-18	4.28, **m**
C-18^1 (-CH_3)	23.7	H-18^1	1.68, **d**, *7.3*
C-19	**		
C-20 (-CH=)	95.7	H-20	8.51, **s**

(*Continued*)

Table 2. (*Continued*)

Triazole and spacer ("**Spr**")					
^{13}C	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	at **N13²**	at **N3²**		at **N13²**	at **N3²**
C-1 (>N-***C***H$_2$-C<)	35.7	42.9 42.8*	H-1[a] H-1[b]	5.69, **d**, *15.0* 5.59, **d**, *15.0*	4.07, 4.01*, **d**, *15.0* 4.04, 4.00*, **d**, *15.0*
C-2	145.8	146.5			
C-3 (-CH=)	125.8	124.6	H-3	8.13, **s**	7.91, **s**
C-7	52.7	52.4	H-7[a] H-7[b]	5.26, **d**, *16.9* 5.23, **d**, *16.9*	5.24, **d**, *16.9* 5.23, **d**, *16.9*
C-8 (>C=O)	167.1	167.0			
			NH-9	7.83, **t**, *5.6*	7.78, **t**, *5.6*
C-10	43.6	43.6	H-10[a] H-10[b]	3.96, **dd**, *16.5*, *5.5* 3.86, **dd**, *16.5*, *5.5*	3.91, **dd**, *16.5*, *5.5* 3.82, **dd**, *16.5*, *5.5*
C-11 (>C=O)	169.6	169.5			
			NH-12	7.85, **d**, *9.6*	7.74, **d**, *9.6*
β-D-galactopyranose **("Gal")**					
^{13}C	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	at **N13²**	at **N3²**		at **N13²**	at **N3²**
C-1	101.3	101.4	H-1	4.76, **d**, *7.7*	4.81, **d**, *8.0*
C-2	69.8	69.9	H-2	5.01, **dd**, *10.4*, *7.7*	5.04, **dd**, *10.3*, *8.0*
C-3	71.7	71.7	H-3	5.05, **dd**, *10.4*, *3.3*	5.05, **dd**, *10.3*, *3.3*
C-4	67.9	67.9	H-4	5.32, **d**, *3.3*	5.32, **d**, *3.3*
C-5	71.2	71.2	H-5	4.20, **t**, *7.0*	4.20, **t**, *7.0*
C-6	61.7	61.7	H-6[a] H-6[b]	4.12, **m** 4.09, **m**	4.12, **m** 4.09, **m**
OAc (-***C***O-)	169.0–171.0				
OAc (-***C***H$_3$)	20.0–21.5		OAc	1.17–2.30, **s**	
β-D-glucopyranose **("Glc")**					
^{13}C	δ, ppm		^{1}H	δ, ppm, multiplicity, *J*, Hz	
	at **N13²**	at **N3²**		at **N13²**	at **N3²**
C-1	78.3	78.5	H-1	5.26, **t**, *9.5*	5.34, **t**, *9.5*
C-2	71.6	71.7	H-2	4.80, **t**, *9.4*	4.86, **t**, *9.5*
C-3	73.5	73.7	H-3	5.22, **t**, *9.5*	5.28, **t**, *9.5*
C-4	76.8	76.9	H-4	3.85, **t**, *9.5*	3.90, **t**, *9.5*
C-5	75.1	75.2	H-5	3.86, **m**	3.90, **m**
C-6	63.1	63.1	H-6[a] H-6[b]	4.38, **m** 4.11, **m**	4.44, **m** 4.14, **m**
OAc (-***C***O-)	169.0–171.0				
OAc (-***C***H$_3$)	20.0–21.5		OAc	1.17–2.30, **s**	

* Signals of two diastereomers. ** The signals are unassigned.

and supplemented with 8–10% fetal calf serum (FCS), 2 mM L-glutamine (Scientific and production enterprise (NPP) "Paneko", Russia). The cell line was provided by D.I.Ivanovsky Institute of virology of the Russian Academy of medical sciences.

Photodynamic effect *in vitro*

For survival assays, cells were seeded into a 96-well flat-bottom microplate ("Costar", USA), 100 μL of the cell suspension (0.7×10^5 cells/mL) per well. Experiments were started 24 h after placing the cells. The compounds were dissolved using 10% Cremophor EL (Sigma). The concentration of the solutions was 1 mg/mL. Further, two-fold sequential dilutions of the photosensitizers in the Eagle's minimum essential medium from 0.03 μM to 5 μM were prepared and placed into wells containing the cells. To assess phototoxicity, cells were incubated with dyes for 2 h and then irradiated with a halogen lamp (500 W) through a KS-19 broadband filter ($\lambda > 720$ nm) and a water filter 5 cm thick. The power density was 12.5–13.0 mW/cm^2; the calculated light dose was 10 J/cm^2.

After the irradiation, the cells were incubated for 24 h under standard conditions. Cells pre-incubated with a photosensitizer without exposure to light, cells irradiated with red light in the absence of any dye, as well as non-exposed cells were used as blank specimens.

To assess cytotoxic activity, cells were kept for 24 h under dark conditions. The survival of cells was assessed by the colorimetric method using the MTT test [23]. To perform a comparative assessment of phototoxicity based on three successive tests, the photosensitizer concentration causing a 50% inhibition of the culture growth (IG_{50}) was calculated.

RESULTS AND DISCUSSION

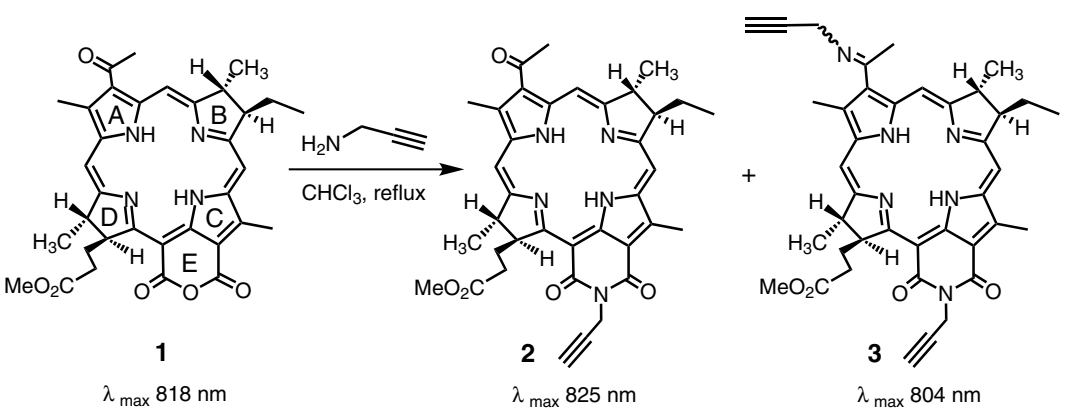
Previously glycoconjugated chlorophyll derivatives have been synthesized using a "click chemistry" [24]. This approach unites simple and facile chemical reactions for the creation of new carbon-carbon and carbon-heteroatom bonds. Click reactions are irreversible, occur under mild conditions and give target products in high yields; therefore, they are used to create supramolecular structures (bioconjugates) in bioorganic chemistry, for immobilization of biomolecules on various types of supports, and for targeted drug delivery.

The following reactions in organic synthesis belong to the above reaction type [25, 26]:

— selected 1,3-dipolar cycloaddition and hetero-Diels–Alder reactions;
— nucleophilic opening of strained heterocycles in epoxides and aziridines;
— reactions of non-aldol carbonyl compounds, including the formation of oxime ethers, hydrazones and aromatic heterocycles.

Of all the above types of chemical reactions, [3+2]-cycloaddition of terminal alkynes and azides to give a substituted triazole [27] is widely used. The azide and alkynyl groups can be readily incorporated into organic molecules; they are stable and inert toward other functional groups in biomolecules. The use of copper(I) compounds as the catalyst in this reaction allowed a highly efficient method for regioselective preparation of 1,4-disubstituted 1,2,3-triazoles to be developed [28]. It has been shown in our previous study that the above reaction between chlorins with a terminal triple bond and sugar azides is an efficient method to synthesize carbohydrate-containing pigments [24].

Here we outline the synthesis of glycoconjugated bacteriochlorophyll derivatives using *N*-propargyl-bacteriopurpurinimide methyl ester (**2**) as the alkynyl bacteriochlorin derivative; the former was synthesized from bacteriopurpurin as we reported previously [18]. A study of this reaction has now shown that, along with the target product **2**, a Schiff base **3** is formed in the reaction of propargylamine with the acetyl group in pyrrole A (Scheme 1). By varying the amount of propargylamine



Scheme 1. Reaction of bacteriopurpurin methyl ester with propargylamine

and the reaction time, one can affect the ratio of products **2** and **3**. In fact, refluxing bacteriopurpurin methyl ester (**1**) with an eightfold molar excess of propargylamine in chloroform for 16 h gave cycloimide **2** in 60% yield and Schiff base **3** in 25% yield. Increasing the excess of propargylamine to 40 equiv. and the reaction time to 40 h resulted in the predominant formation of dipropargyl derivative **3**.

The mass spectra of the resulting compounds **2** and **3** contain the corresponding molecular ion peaks. The IR spectrum contains an intense stretching vibration band of the C–H bond at 3300 cm^{-1} and a band at lower frequencies (about 2100 cm^{-1}) corresponding to the stretching vibrations of the carbon-carbon bond in the terminal acetylene moiety C≡CH. It is interesting to note that the absorption maximum of compound **3** in the electronic spectrum is shifted by 20 nm to shorter wavelengths (804 nm) in comparison with cycloimide **2**, which indirectly confirms the formation of a Schiff base in pyrrole ring A. The ^{1}H NMR spectra of the resulting compounds **2** and **3** contain proton signals of propargyl residues. In fact, the spectrum of cycloimide **2** contains a triplet of the terminal alkynyl proton at $\delta = 2.30$ ($J = 2.4$) and signals of protons of the methylene group in the form of an ABX system ($\delta_A = 5.22$, $\delta_b = 5.27$, $J_{AB} = 16.5$ Hz, $J_{AX} = 2.4$, $J_{BX} = 2.4$).

Cycloimide **2** was brought into reaction with peracetylated *N*-(*N*-azidoacetylglycyl)-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (**4**), which was obtained by *N*-acylation of 4-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine with succinimide azidoacetate followed by *O*-acetylation of hydroxy groups with acetic anhydride [29]. Succinimide azidoacetate was synthesized by the general method for the preparation of activated esters by condensation of azidoacetic acid and *N*-hydroxysuccinimide with *N,N′*-dicyclohexylcarbodiimide (DCC) in 1,2-dimethoxyethane [30].

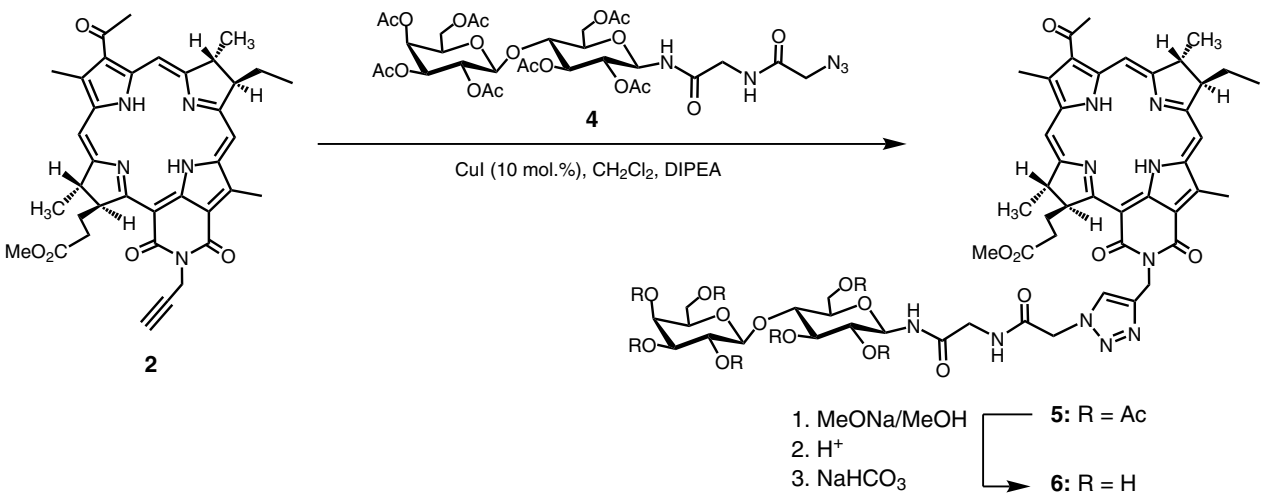
The click reaction was carried out using 10 mol.% of copper(I) iodide in dichloromethane with an addition of diisopropylethylamine (DIPEA). Previous experience in performing a similar reaction in a series of chlorins has shown that the Cu^+ cation is readily incorporated into the macrocycle, which results in a catalyst loss. Therefore, we implemented an approach to the metal-free glycoconjugate based on the use of a Zn complex.

It has been shown that the latter is sufficiently stable under the reaction conditions and no transmetallation occurs. Such "protection" by the Zn^{2+} cation followed by demetallation in a weakly-acidic medium allowed us to obtain a metal-free glycosylated chlorin e_6.

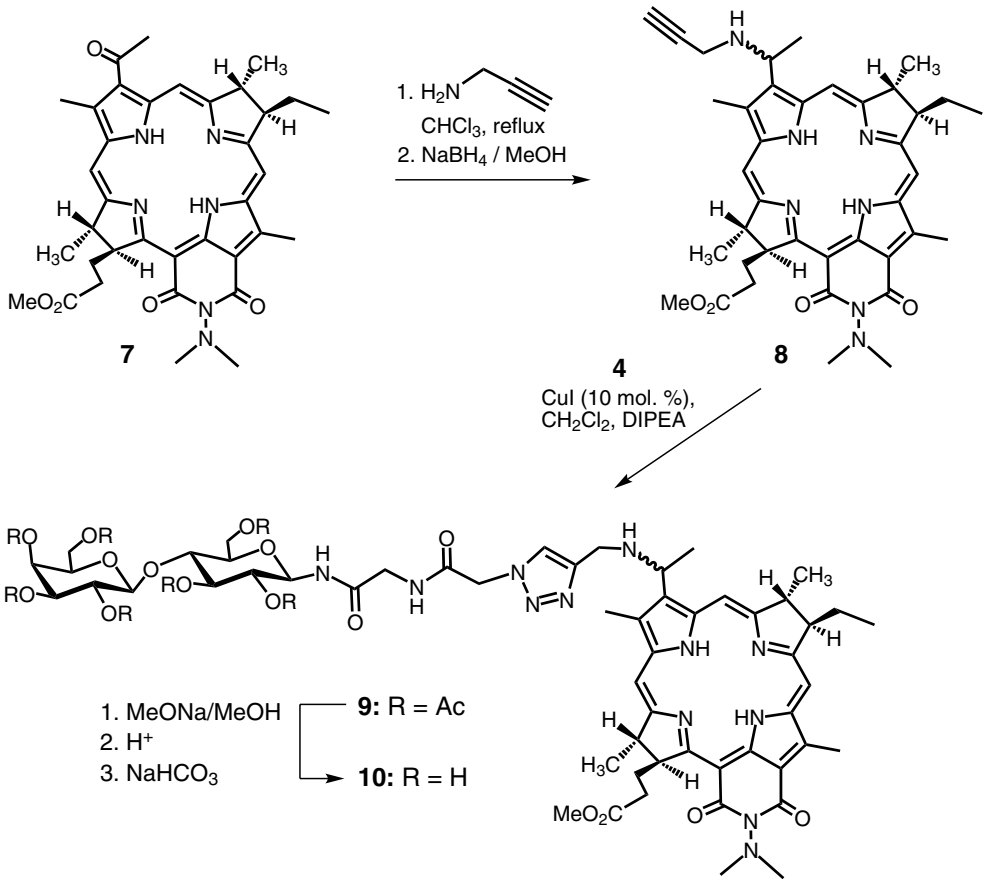
No formation of the Cu-bacteriochlorin cycloimide complex in the reaction was observed in this study, which agrees with the fact well-known in porphyrin chemistry that the facility of metal complex formation decreases in the porphyrins-chlorins-bacteriochlorins series. The reaction was completed in 20 min at room temperature to give glycoconjugate **5** in 80% yield. Deprotection of lactose hydroxyl groups with a NaOMe/MeOH solution gave conjugate **6** in a high yield.

Regioselective attachment of sugar to pyrrole ring A was carried out using *N*-dimethyl-amino-bacteriopurpurinimide **7** as the starting compound; we have described the synthesis and properties of this compound previously [21, 31]. Refluxing of compound **7** with propargylamine in chloroform resulted in the corresponding Schiff base, which was reduced with sodium borohydride. The resulting mixture of diastereomeric propargyl derivatives **8** was coupled with the lactose peracetate azide derivative **4**.

As a result, glycoconjugate **9** was obtained in 83% yield. Deprotection of hydroxyl groups in compound **9** resulted in a new lactosyl derivative of bacteriochlorin *p* cycloimide **10**; its enhanced hydrophilicity and the presence of a carbohydrate vector will probably improve



Scheme 2. Synthesis of bacteriochlorin *p* cycloimide conjugate with a lactose azide derivative



Scheme 3. Incorporation of a lactose azide derivative at pyrrole ring A of cycloimide bacteriochlorin *p*

the photodynamic efficiency of *N*-dimethylamino-bacteriopupurinimide [7].

It is well-known that an increase in the number of carbohydrate moieties in glycoconjugates enhances the efficiency of binding with galectins. With this in mind, we implemented a synthesis of a bivalent bacteriochlorin lactosyl derivative based on purpurinimide **3**. The latter was reduced with sodium borohydride to give a mixture of diastereomeric propargylamines **11**, which were then coupled with lactose azide derivative **4** (2 equiv.) (Scheme 4) to give conjugate **12** in 70% yield; deprotection of hydroxy groups gave product **13** with enhanced hydrophilicity, which was partially soluble in water.

NMR analysis of glycoconjugates

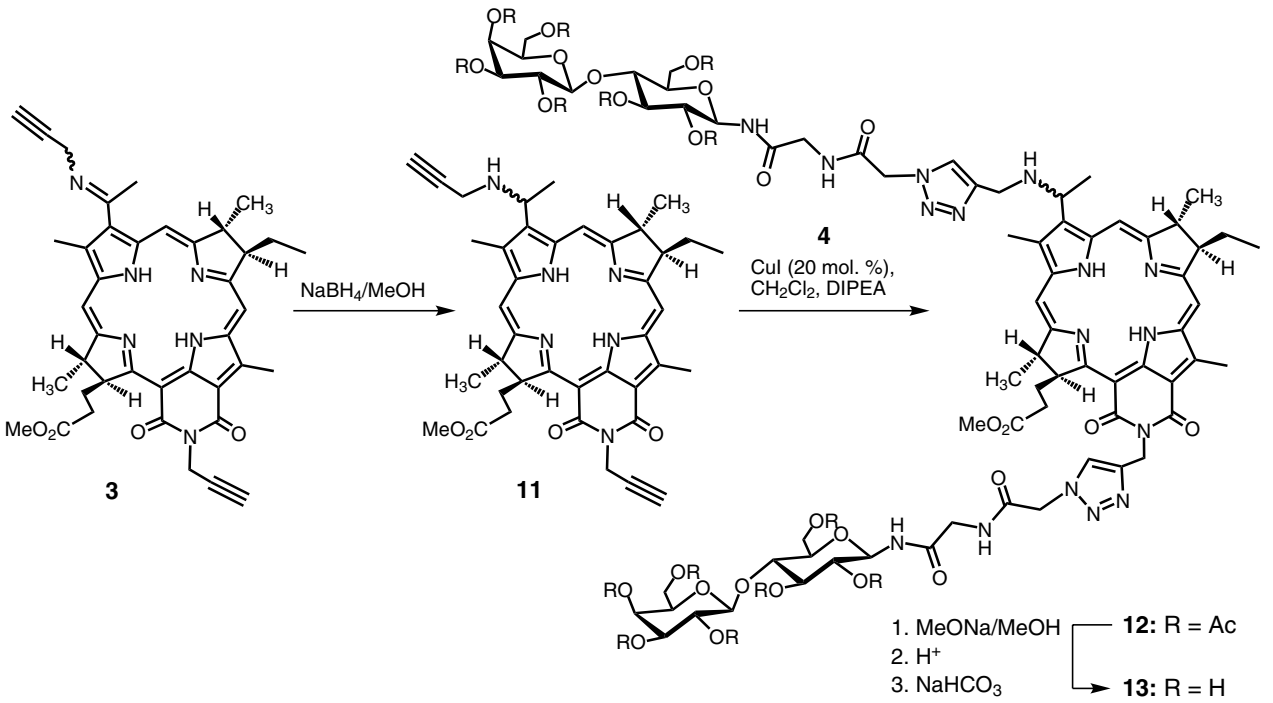
The structure of compounds **5**, **9**, and **12** was elucidated by 1D and 2D NMR spectra for ^{1}H, ^{13}C, and ^{15}N. The ^{1}H and ^{13}C NMR spectra were assigned using 2D homonuclear ^{1}H-^{1}H gCOSY, TOCSY and ROESY and heteronuclear ^{1}H-^{13}C gHSQC, gHMBC experiments. The signals of three parts of the molecule, namely bacteriochlorin, lactose and the triazole-containing spacer, were assigned.

Analysis of homonuclear 2D spectra helped us to assign several isolated spin systems. TOCSY spectra revealed 4-substituted β-glucopyranose and β-galactopyranose protons, isolated CH_2 and HN–CH_2 groups in the spacer and bacteriochlorin coupled protons H-7, H-7^1; H-8, H-8^1, H-8^2; H-17, H-17^1, H-17^2; H-18, H-18^2, as well as isolated methyl (H-2^1, H-12^1) and *meso*-protons (H-5, H-10, H-20). The COSY spectrum enabled differentiation between protons within each spin system. Their carbon atoms were assigned using ^{1}H-^{13}C gHSQC spectra.

The connectivity between sugar residues, spacer, and bacteriochlorin was determined from ^{1}H-^{1}H ROESY, ^{1}H-^{13}C and ^{1}H-^{15}N gHMBC spectra.

The analysis is described using compound **5** as an example. In the ROESY spectrum, we found correlations between the anomeric proton of β-galactose (4.47 ppm) with H-4 of β-glucose (3.77 ppm) that corresponds to the lactose disaccharide. The amide group NH-9 (7.13 ppm) and H-12 (7.07 ppm) protons from the spacer had ROESY correlations with H-7 (5.22 ppm), H-10 (3.92, 3.83 ppm) and H-1(β-Glc) (5.13 ppm), H-2(β-Glc) (4.84 ppm), correspondingly (Fig. 1).

These correlations confirm the structure of the spacer moiety, as well as connectivity between the spacer and



Scheme 4. Synthesis of bivalent lactosylbacteriopurpuruinimide

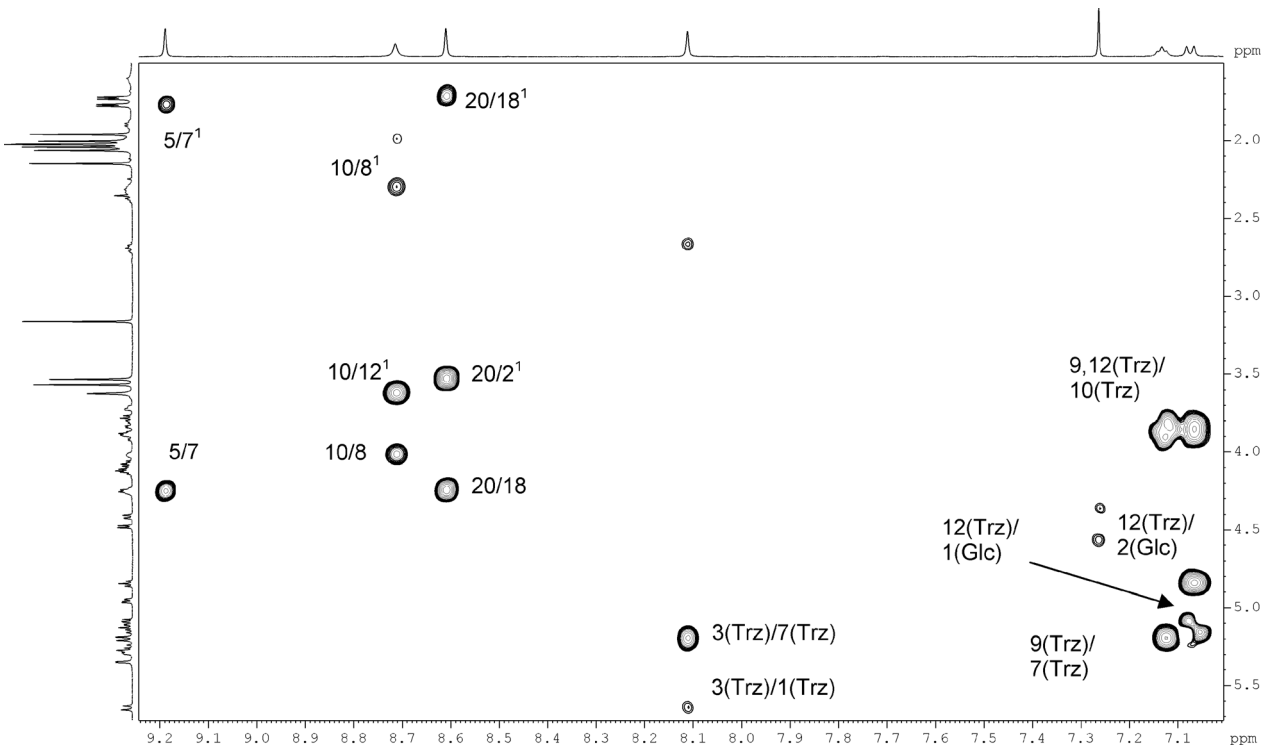


Fig. 1. Selected ROESY correlations for compound **5**

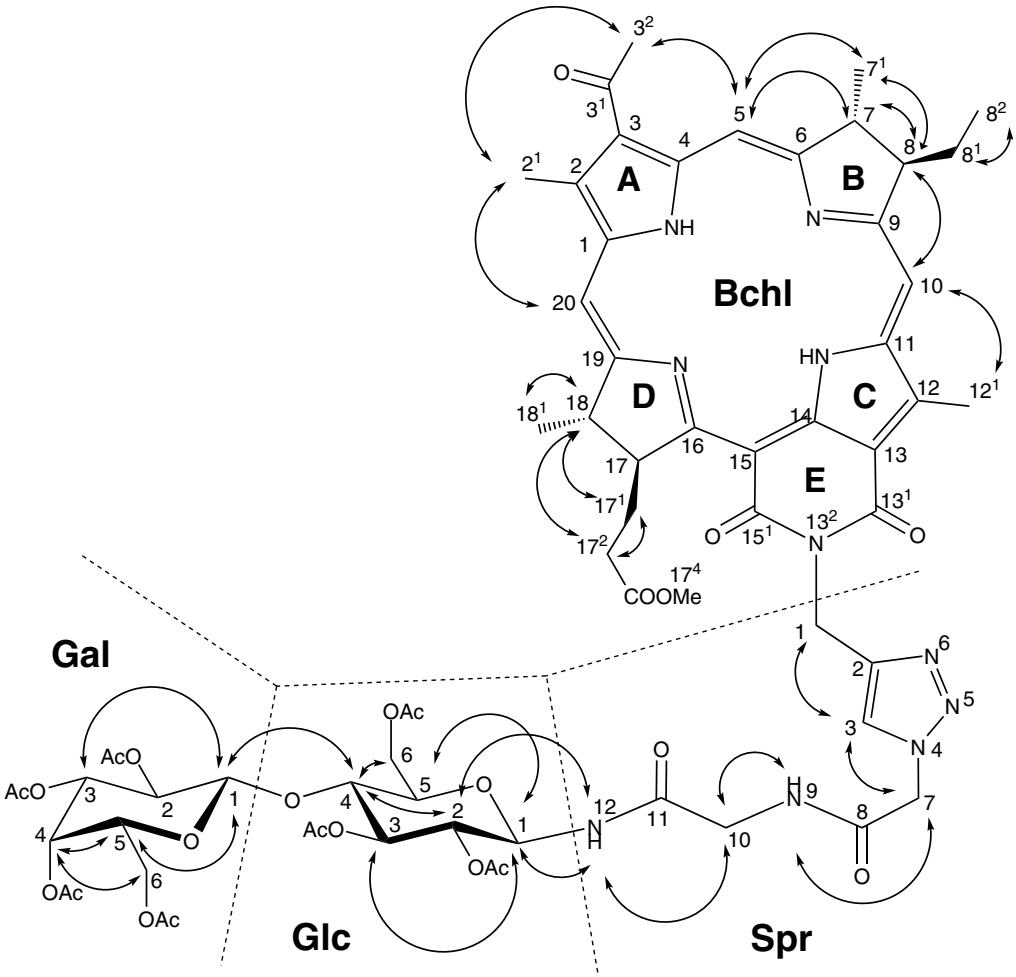


Fig. 2. Structure of conjugate **5**. Dashed lines separate the residues: Bacteriochlorin *p* (**Bchl**), Triazole and spacer (**Spr**), β-D-Glucopyranose (Glc), β-D-Galactopyranose (Gal). Observed ^{1}H-^{1}H NOEs are marked with two-headed arrows, including those not mentioned in the text

lactose (Fig. 2). On the other hand, ROESY correlations helped us to assign selected groups in bacteriochlorin, using the H-5, H-10, and H-20 protons.

This data was supported by a ^{1}H-^{13}C HMBC spectrum, where correlations between the protons and the corresponding carbons were found. Furthermore, this spectrum helped us to discover all quaternary carbon atoms in bacteriochlorin and triazole moieties, as well as O-acetyl groups in lactose. Thus, the ^{13}C spectrum was completely assigned.

Since these molecules contain nitrogen atoms that may also play a role in the structure elucidation, we performed a ^{1}H-^{15}N HMBC correlation experiment to reveal proton-nitrogen one- and multiple-bond interactions (Fig. 3).

Due to good experimental conditions, we obtained all requested correlations that allowed us to assign all nitrogen atoms in the molecule and their connectivity to protons.

All spectral data obtained from hetero- and homonuclear 2D correlations had no contradictions as concerns the atom/bond assignment. For compounds **9** and **12** we observed signals from two diastereomers, which had a noticeable difference in the chemical shifts for ring A of the bacteriochlorin and triazole, as well as some minor differences in glucose and other rings of the bacteriochlorin.

Thus, the data obtained allowed us to establish the structures and to study the intramolecular spatial contacts in compounds **5**, **9** and **12**; furthermore, the selective formation of a 1,4-disubstituted triazole ring was confirmed.

Analysis of absorption and fluorescence spectra of photosensitizers 6, 10, 13

Analysis of absorption spectra of bacteriochlorin *p* glycosylated derivatives has shown that all the compounds studied are stable for 2 h of incubation in Eagle's minimum essential medium with phenol red and supplemented with 8–10% fetal calf serum (FCS). No changes in the spectrum profile or in optical absorption of all the dyes studied were observed within the time range used.

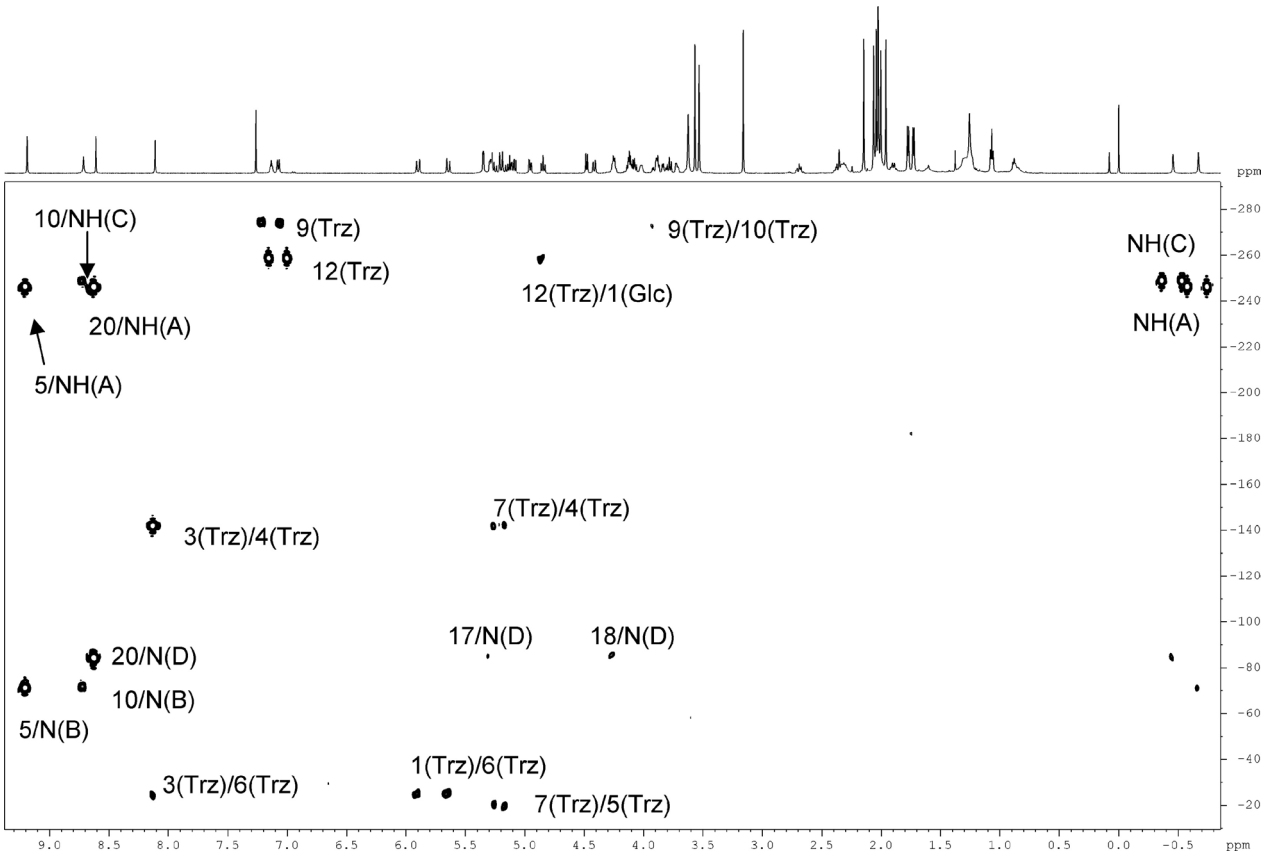


Fig. 3. The ^{1}H-^{15}N gHMBC spectrum of compound **5** (H/N)

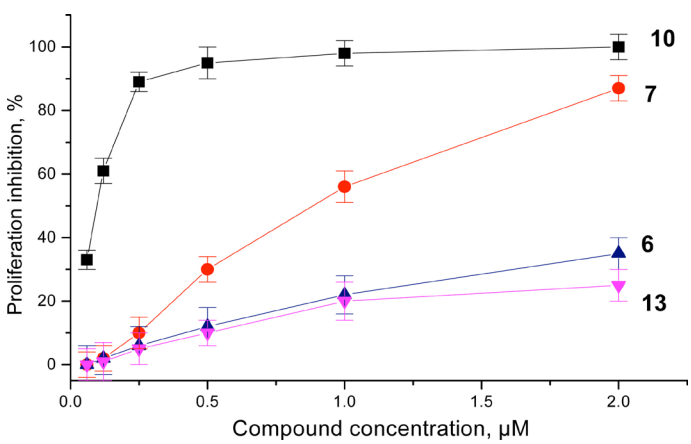


Fig. 4. Photoinduced activity of photosensitizers **6, 10, 13** and **7** *versus* concentration in the incubation medium

In vitro activity

The photoinduced activity of bacteriopurpurinimide derivatives with carbohydrate substituents at various macrocycle positions (photosensitizers **6**, **10** and **13**) was studied using human tumor cells of epithelial origin (cell line HEp2). Purpurinimide **7** involving no carbohydrate fragment was used for comparison.

The experiments have shown that the position of the disaccharide residue at the macrocycle affects considerably the activity of photosensitizers (Figs 4 and 5).

In fact, of all the compounds studied, the maximum photoinduced activity was shown by the dye (**10**) with a disaccharide residue at pyrrole A (IG_{50} = 90 nM); the

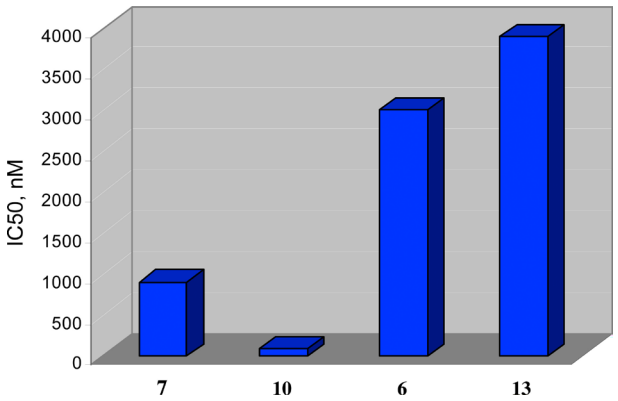


Fig. 4. IC50 unconjucated cycloimide bacteriopurpurinimide (**7**) and glycoconjugates (**6**, **10**, **13**)

unconjugated cycloimide bacteriopurpurinimide (**7**) also showed some phototoxicity (IG_{50} = 900 nM). It should be noted that conjugates **6** and **13** had low photoinduced activity towards HEp2 cells (the inhibition of cell proliferation, taking dark toxicity into account, did not exceed 25–35%).

CONCLUSION

Thus, we have shown in this paper that the click reaction is perfect for synthesizing glycoconjugates based on such sufficiently labile compounds as bacteriochlorophyll *a* derivatives. The mild reaction conditions and high product yields allow this reaction to be used in the large-scale preparation of carbohydrate-containing PS for targeted PDT of cancer.

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REFERENCES

1. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J and Peng Q. *J. Natl. Cancer I.* 1998; **90**: 889–905.
2. Jori G. *Photochem. Photobiol*. 1990; **52**: 439–443.
3. Bonnett R. *Chem. Soc. Rev*. 1995; **24**: 19–33.
4. Dolphin D. *Can. J. Chem.* 1994; **72**: 1005–1013.
5. Grin MA, Mironov AF and Shtil AA. *Anti-Cancer Agents Med. Chem.* 2008; **8**: 683–697.
6. Barondes SH, Castronovo V, Cooper DNW, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu F, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirer F, Raz A, Rigby PWJ, Rini JM and Wang JL. *Cell*. 1994; **76**: 597–599.
7. Zheng G, Graham A, Shibata M, Missert JR, Oseroff AR, Dougherty TJ and Pandey RK. *J. Org. Chem*. 2001; **66**: 8709–8716.
8. Park YK, Bold B, Cui BC, Bai JQ, Lee W and Shim YK. *Bull. Korean Chem. Soc*. 2008; **29**: 130–134.
9. Zheng X and Pandey RK. *Anti-Cancer Agents Med. Chem.* 2008; **8**: 241–268.
10. Chen X, Hui I, Foster DA and Drain CM. *Biochemistry*. 2004; **43**: 10918–10929.
11. Aksenova AA, Sebyakin YL and Mironov AF. *Russ. J. Bioorg. Chem*. 2003; **29**: 201–219.
12. Laville I, Pigaglio S, Blais JC, Doz F, Loock B, Maillard PH, Grierson DS and Blais J. *J. Med. Chem*. 2006; **49**: 2558–2567.
13. Li G, Pandey SK, Graham A, Dobhal MP, Mehta R, Chen Y, Gryshuk A, Rittenhouse-Olson K, Oseroff A and Pandey RK. *J. Org. Chem*. 2004; **69**: 158–172.
14. Mikata Y, Onchi Y, Shibata M, Kakuchi T, Ono H, Ogura S, Okura I and Yano S. *Bioorg. Med. Chem. Lett*. 1998; **8**: 3543–3548.
15. Laville I, Figueiredo T, B. Loock B, Pigaglio S, Maillard PH, Grierson DS, Carrez D, Croisy A and Blais J. *Bioorg. Med. Chem*. 2003; **11**: 1643–1652.
16. Silver AMG, Tomer AC, Neves MGPMS, Silva AMS, Cavaleiro JAS, Perrone D and Dondoni A. *Tetrahedron Lett*. 2002; **43**: 603–605.
17. Mironov AF and Grin MA. *J. Porphyrins Phthalocyanines* 2008; **11**: 1163–1172.
18. Grin MA, Lonin IS, Lakhina AA, Ol'shanskaya ES, Makarov AI, Sebyakin YL, Guryeva Lyu, Toukach PV, Kononikhin AS, Kuzmin VA and Mironov AF. *J. Porphyrins Phthalocyanines* 2009; **13**: 336–345.
19. Dumoulin F and Ahsen V. *J. Porphyrins Phthalocyanines* 2011; **15**: 481–504.
20. Mironov AF, Kozyrev AN and Brandis AS. *Proc. SPIE.* 1993; **1922**: 204–209.
21. Mironov AF, Grin MA, Tsiprovskiy AG, Kachala VV and Karmakova TA. *J. Porphyrins Phthalocyanines* 2003; **7**: 725–730.
22. Gottlieb HE, Kotlyar V and Nudelman A. *J. Org. Chem*. 1997; **62**: 7512–7515.
23. Carmichael J, DeGraff WG, Gazdar AF, Minna JD and Mitchell JB. *Cancer Res*. 1987; **47**: 936–942.
24. Grin MA, Lonin IS, Makarov AI, Lakhina AA, Toukach FV, Kachala VV, Orlova AV and Mironov AF. *Mendeleev Commun.* 2008; **18**: 135–137.
25. Kolb HC, Finn MG and Sharpless KB. *Angew. Chem. Int. Ed. Engl*. 2001; **40**: 2004–2021.

26. Kolb HC and Sharpless KB. *Drug Discovery Today.* 2003; **8**: 1128–1137.
27. Huisgen R. *1,3-Dipolar Cycloadditional Chemistry*, Padwa A. (Ed.) Wiley: New York, 1984.
28. Tornoe CW, Christensen C and Meldal M. *J. Org. Chem*. 2002; **67**: 3057–3064.
29. Likhosherstov LM, Novikova OS, Zheltova AO and Shibaev VN. *Russ. Chem. Bull.* 2000; **49**: 1454–1459.
30. Anderson GW, Zimmerman JE and Callahan FM. *J. Am. Chem. Soc*. 1964; **86**: 1839–1842.
31. Sharonov GV, Karmakova TA, Kassies R, Pljutinskaya AD, Grin MA, Refregiers M, Yakubovskaya RI, Mironov AF, Maurizot JC, Vigny P, Otto C and Feofanov AV. *Free Radical Biol. Med.* 2006; **40**: 407–419.