A Straightforward and Mild Method of Tethering Monosaccharides to Thieno[2,3-*d*]pyrimidinones *via* the Copper(I)-catalyzed Azide-Alkyne 'Click Chemistry'

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Z. Naturforsch. **2012**, *67b*, 1282 – 1288 / DOI: 10.5560/ZNB.2012-0193
Received July 8, 2012

A mild and versatile method based on Cu(I)-catalyzed [3 + 2] cycloaddition (Meldal-Sharpless reaction) was developed to tether biomolecules, such as monosacharides or lipophylic azides, to alkyne functions of spirobenzo[*b*]thieno[2,3-*d*]pyrimidine-1′-cyclohexane. The reactions are highlighted by their modularity and high efficiency with excellent yields in most cases. The products are interesting precursors for a variety of applications.

Key words: Thienopyrimidinone, Alkynes, Azides, Click Chemistry

Introduction

The construction of structures of increasing size and often corresponding complexity from modular components not only lies at the heart of synthetic chemistry, but is also the basis of many biological processes essential to life [1 – 4]. An excellent example of this is the 'click chemistry' which represents a modular approach toward syntheses and use only the most practical chemical transformations to make molecular connections with excellent fidelity [5 – 7]. The 1,3-dipolar cycloaddition of azides and alkynes (AAC) to give substituted 1,2,3-triazoles has emerged as a powerful linking reaction in both uncatalyzed [8] and metal-catalyzed [9] forms (Fig. 1).

The practical importance of the process derives from the easy introduction of azide and alkyne functions into organic compounds and the stability of these groups toward other reaction conditions. The copper-catalyzed (CuAAC) version has proven to be popular in applications ranging from drug discovery [10] to surface science [11], where rapid and reliable bond formation is required. The significant growth of 'click chemistry' and in particular the copper-catalyzed azide-alkyne cycloaddition reaction [12, 13] (CuAAC) in the fields of macromolecular and surface science highlights the fundamental necessity for a core group of reproducible and broadly applicable reactions which may be employed across diverse disciplines of the physical and biological sciences. Among promising targeting therapies for cancer treatment, substituted thienopyrimidones have continued to retain attention of both academic institutions and pharmaceutical companies in the last few years [14 – 16]. To the best of our knowledge thieno[2,3-*d*]pyrimidinones have never been conjugated with saccharides using a click reaction. Only very recently we became interested in the application of the copper-catalyzed cycloaddition of azides and alkynes to pyridothienopyrimidinone [11, 17] systems in order to tether carbohydrates and amino acids to this pharmacophor *via* 1,2,3-triazole linkers for novel anticancer therapeutics or nanoscaled molecular rods.

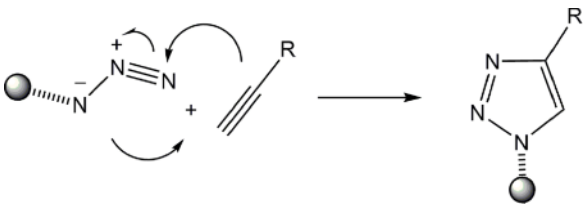


Fig. 1. 1,3-Dipolar cycloaddition of azides and alkynes (AAC).

As an extension of this work we now report the preparation of novel spirothienopyrimidinones which

have the advantage of introducing multiple biological functionalities *via* conjugation with biomolecules using 'click chemistry'.

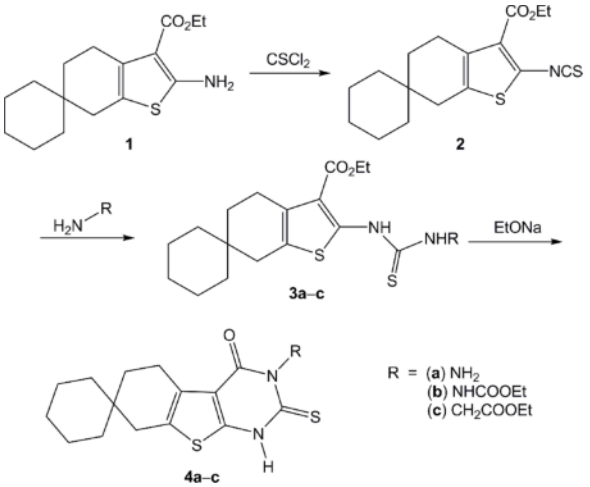
Results and Discussion

The synthesis of 3-substituted-2-thioxo-4-oxo-3,4,5,8-tetrahydro-spirobenzo[*b*]thieno[2,3-*d*]pyrimidine-7(6*H*),1′-cyclohexane (**4a – c**) to construct the target *S*-propargylated pyrimidinone **5** is summarized in Scheme 1. At first, the reaction of the readily available spiro[5,5]undecane-3-one [18 – 21] with ethyl cyanoacetate and sulfur in absolute ethanol and morpholine, in analogy to Gewald [22] procedures, gave the 2-amino-4,7-dihydro-spiro[benzo[*b*]thiophene-6(5*H*),1′-cyclohexane]-3-carboxylic acid ethyl ester **1**, which was converted into the isothiocyanate product **2** by treatment with thiophosgene. The key intermediates **3a – c** could be obtained by treatment of the isothiocyanate **2** with hydrazine hydrate, ethyl carbazate or glycine ethyl ester hydrochloride in the presence of triethylamine with a good yield. Base-catalyzed cyclization of compounds **3a – c** followed by reprotonation of the obtained sodium salt by HCl produced the thione precursors **4a – c** in excellent yields.

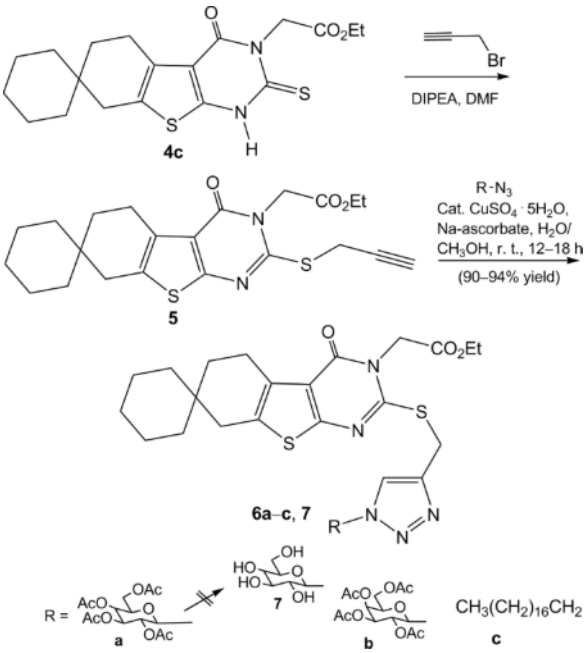
Propargylation of the SH group in compound **4c** with propargyl bromide in the presence of diisopropylethylamine gave rise to the product **5** (Scheme 2). The position of the propargyl group in **5** was elucidated by ^{13}C NMR spectroscopy ($\delta = 54.2$, 72.2 and 77.7 ppm for HCC-*C*H$_2$) with absence of a C=S signal which appeared at $\delta = 174.0$ ppm in compound **4c**. In addition the ^{1}H NMR spectrum of **5** showed a broad singlet and doublet at $\delta = 2.18$ and 3.96 ppm for the CH and CH$_2$ units of the propargyl group, respectively.

Making use of the alkyne function in compound **5** we seeked to tether different monosaccharides *via* 1,2,3-triazoles. Under the broadly known copper-catalyzed alkyne/azide click (CuAAC) reaction conditions, reacting compound **5** with 1-azido-1-deoxy-2,3,4,6-tetraacetyl-β-D-hexoses (glucose and galactose) using CH$_3$OH-H$_2$O (1:1) as a solvent mixture produced the cycloaddition products **6a**,**b** in excellent yields (Scheme 2). Based on these results we applied a similar methodology to produce the octadecyl-1,2,3-triazolo product **6c** by reacting the alkyne **5** with octadecyl azide.

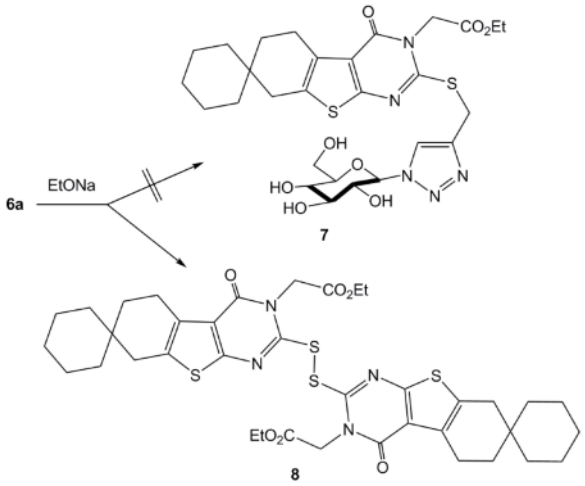
The 1,2,3-triazole-bearing thienopyrimidinone conjugates **6a – c** appeared as colorless, stable solids. Their structures were confirmed by spectroscopic methods. In addition to the signals of the thienopyrimidinone, the biomolecules, and the linker moieties, diagnostic signals for the 1,2,3-triazole ring were found in the NMR spectra [$\delta = 7.7 - 7.8$ (^{1}H NMR), $\delta = 120 - 122$ and 142 – 146 ppm (^{13}C NMR)].



Scheme 1. Synthesis of the pyrimidinone derivatives **4a – c**.



Scheme 2. Synthesis of the clicked products **6a – c**.



Scheme 3. Synthesis of the disulfide product **8**.

Although homodisulfides are generally synthesized *via* oxidative coupling of their corresponding thiols [23], many procedures are offered for this conversion including using halogen-containing reagents [24], metal ions [25] and hydrogen peroxide [26]. Sucholeiki [27] reported that thioethers can readily be used for the attachment of organic molecules to solid supports and can undergo light-induced heterogeneous C–S bond cleavage upon irradiation with 350 nm light.

Remarkably, removal of the acetyl groups from the sugar units of **6a** to produce compound **7** turned out to be difficult in the presence of unstoichiometric amount of sodium methoxide (0.1 equiv.). Only a mixture of products was obtained under these conditions containing traces of the product. The reaction was repeated with 0.3 equiv. of sodium ethoxide to avoid transesterification. UPLC analysis revealed that only the disulfide product **8** was isolated as a solid which was characterized by HRMS and NMR spectroscopy (Scheme 3).

Conclusion

In the present context, the copper-catalyzed 1,3-dipolar cycloaddition (CLICK) reaction was shown to be an ideal choice as it usually proceeds in high yield under mild conditions. We adopted a convergent strategy, in which a preformed alkyne core functionalized with the desired thieno[2,3-*d*]pyrimidinone could be conjugated with saccharides and a lipophilic azide. A novel heterocyclic disulfide system was obtained in an unusual conversion. All products obtained were hitherto unknown.

Experimental Section

General Remarks: All reactions were carried out with oven-dried glassware. Solvents were dried. Starting materials were purchased from Aldrich and Merck. Azides of monosaccharides and octadecanyl azide were synthesized according to literature procedures [28, 29]. TLC analysis was performed on Merck silica gel 60 F254 plates and visualized by UV illumination and by charring with phosphomolybdic acid, potassium permanganate or ninhydrin. Silica gel 60 (0.035 – 0.070 mm, Acros) was used for preparative column chromatography. Melting points were determined on a Boetius hotstage apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively, on a Bruker AC-300 with TMS as internal standard. High-resolution mass spectra (ESI) were measured with a Thermo Finnigan LTQ-FT-ICR-MS with MeOH as solvent.

*Synthesis of 2-isothiocyanato-4,7-dihydrospiro[benzo[b]-thiophene-6(5H),1′-cyclohexane]-3-carboxylic acid ethyl ester (**2**)*

A suspension of compound **1** (0.60 g, 2 mmol) in CH_2Cl_2 (5 mL) was added to a stirred suspension of calcium carbonate (1.8 g, 18 mmol) in water (10 mL) and CH_2Cl_2 (20 mL) at room temperature. To the stirred mixture, thiophosgene (0.24 g, 2 mmol) was added slowly in an ice bath. The temperature of the reaction mixture was allowed to reach room temperature. Stirring was continued over night. Inorganic salts were removed by filtration, and then the organic phase was washed with water (2 × 20 mL), 5 % aqueous sodium bicarbonate (2 × 20 mL) and brine (30 mL). After drying over magnesium sulfate, the solvent was removed under vacuum, and the residue was purified by column chromatography to give yellow crystals (0.42 g, 73 %); m. p. 124 – 125 °C. – $R_f = 0.65$ (CH_2Cl_2,100 %). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.29$ (t, 4H, $J = 3.3$ Hz, H-2′, 6′), 1.34 (t, 3H, $J = 7.1$ Hz, $COOCH_2CH_3$), 1.37 – 146 (m, 6H, H-3′,4′,5′), 1.56 (t, 2H, $J = 6.5$ Hz, H-5), 2.39 (s, 2H, H-7), 2.69 (t, 2H, $J = 6.5$ Hz, H-4), 4.29 (q, 2H, $J = 7.2$ Hz, $COOCH_2CH_3$). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.5$ (CH_3), 21.8 (C-3′,5′), 23.0 (C-4), 26.6 (C-4′), 32.5 (C-5), 32.7 (Cq), 35.9 (C-2′,6′), 36.1 (C-7), 60.8 (CH_2, ester), 126.3 (C-7a), 131.4 (C-3a), 132.7 (C=S), 133.8 (C-3), 137.3 (C-2), 162.1 (C=O, ester). – HRMS (ESI): $m/z = 336.1062$ (calcd. 336.1092 for $C_{17}H_{22}NO_2S_2$, $[M+H]^+$).

*Synthesis of **3a – c***

To a solution of the isothiocyanate **2** (2 mmol) in dichloromethane (5 mL), a solution of amine (2 mmol) in

dichloromethane (5 mL) and triethylamine (0.30 g, 3 mmol) were added with stirring. The mixture was stirred for 1 – 2 h, and then the solvent was removed under reduced pressure yielding compounds **3a – c**.

*2-[Hydrazinocarbothioyl]amino-4,7-dihydro-spiro[benzo[b]thiophene-6(5H),1′-cyclohexane]-3-carboxylic acid ethyl ester (**3a**)*

The product was obtained, following the general procedure under stirring at r. t. for 1 h, as a colorless solid product (0.57 g, 91 %); m. p. 198 – 199 °C. – ^{1}H NMR (300 MHz, DMSO): $\delta = 1.29 - 1.43$ (m, 13H, H-2′, 6′, $COOCH_2CH_3$, H-3′,4′,5′), 1.56 (t, 2H, $J = 6.5$ Hz, H-5), 2.41 (s, 2H, H-7), 2.69 (t, 2H, $J = 6.5$ Hz, H-4), 4.29 (q, 2H, $J = 7.2$ Hz, $COOCH_2CH_3$), 4.98 (bs, 1H, *NH*NH_2), 7.16 (bs, 2H, NH_2), 9.64 (bs, 1H, NH). – ^{13}C NMR (75 MHz, DMSO): $\delta = 14.2$ (CH_3), 21.3 (C-3′,5′), 22.8 (C-4), 26.2 (C-4′), 32.1 (C-5), 32.7 (Cq), 34.6 (C-2′,6′), 35.1 (C-7), 60.0 (CH_2, ester), 111.0 (C-7a), 124.2 (C-3a), 128.8 (C-3), 149.3 (C-2), 165.0 (C=O, ester), 176.8 (C=S). – HRMS (ESI): $m/z = 368.1463$ (calcd. 368.1466 for $C_{17}H_{26}N_3O_2S_2$, $[M+H]^+$).

*2-[(Ethoxycarbonyl)-aminocarbothioyl]amino-4,7-dihydro-spiro[benzo[b]thiophene-6(5H),1′-cyclohexane]-3-carboxylic acid ethyl ester (**3b**)*

The product was obtained, following the general procedure under stirring at r. t. for 1 h as colorless solid product (0.78 g, 89 %); m. p. 110 °C (decomposed). – ^{1}H NMR (300 MHz, DMSO): $\delta = 1.23 - 1.42$ (m, 16H, H-2′, 6′, $2COOCH_2CH_3$, H-3′,4′,5′), 1.57 (t, 2H, $J = 6.5$ Hz, H-5), 2.44 (s, 2H, H-7), 2.70 (t, 2H, $J = 6.5$ Hz, H-4), 4.16 (q, 2H, $J = 6.8$ Hz, $NHCOOCH_2CH_3$), 4.30 (q, 2H, $J = 7.2$ Hz, $COOCH_2CH_3$), 9.77 (bs, 1H, *NH*NH), 10.24 (bs, H, NH*NH*), 12.38 (bs, 1H, NH). – ^{13}C NMR (75 MHz, DMSO): $\delta = 14.3$ (CH_3), 14.6 (CH_3), 21.3 (C-3′,5′), 22.7 (C-4), 26.2 (C-4′), 32.2 (C-5), 32.6 (Cq), 34.6 (C-2′,6′), 35.3 (C-7), 60.5 (CH_2, ester), 61.3 (CH_2, ester), 111.8 (C-7a), 125.0 (C-3a), 129.0 (C-3), 148.8 (C-2), 155.8 (C=O, ester), 165.8 (C=O, ester), 177.1 (C=S). – HRMS (ESI): $m/z = 440.1663$ (calcd. 440.1778 for $C_{20}H_{30}N_3O_4S_2$, $[M+H]^+$).

*2-[(2-Ethoxy-2-oxoethyl)-aminocarbothioyl]amino-4,7-dihydro-spiro[benzo[b]thiophene-6(5H),1′-cyclohexane]-3-carboxylic acid ethyl ester (**3c**)*

The product was obtained, following the general procedure under stirring at r. t. for 2 h as a colorless solid product (0.79 g, 90 %); m. p. 139 – 140 °C. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.27 - 1.41$ (m, 16H, H-2′, 6′, $2COOCH_2CH_3$, H-3′,4′,5′), 1.56 (t, 2H, $J = 6.5$ Hz, H-5), 2.45 (s, 2H, H-7), 2.68 (t, 2H, $J = 6.5$ Hz, H-4), 4.16 (q, 2H, $J = 6.8$ Hz, $NHCH_2COOCH_2CH_3$), 4.30 (q, 2H, $J = 7.2$ Hz, $COOCH_2CH_3$), 4.41 (s, 2H, $NHCH_2$), 10.18 (bs, H, NH), 12.54 (bs, 1H, NH). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.3$ (CH_3), 14.5 (CH_3), 21.3 (C-3′,5′), 22.8 (C-4), 26.2 (C-4′), 32.1 (C-5), 32.6 (Cq), 34.5 (C-2′,6′), 35.4 (C-7), 47.9 (CH_2), 60.9 (CH_2, ester), 61.4 (CH_2, ester), 111.8 (C-7a), 124.9 (C-3a), 129.0 (C-3), 148.6 (C-2), 155.7 (C=O, ester), 165.3 (C=O, ester), 176.7 (C=S). – HRMS (ESI): $m/z = 439.1633$ (calcd. 439.1725 for $C_{21}H_{31}N_2O_4S_2$, $[M+H]^+$).

*Synthesis of **4a – c***

A sodium ethoxide solution (0.05 g, 2 mmol in 10 mL of abs. ethanol) was added to the crude corresponding compound **3** (2 mmol). The reaction mixture was stirred at r. t. over night. The formed sodium salt was collected, dissolved in water and the pH of the solution adjusted with hydrochloric acid to pH = 4. The solid product was filtered off, washed with water and recrystallized from ethanol to give compounds **4a – c**.

*3-Amino-2-thioxo-4-oxo-1,2,3,4,5,8-hexahydro-spirobenzo[b]thieno[2,3-d]pyrimidine-7(6H),1′-cyclohexane (**4a**)*

The product was obtained, following the general procedure, as colorless crystals from methanol (0.54 g, 84 %); m. p. 200 – 201 °C. – ^{1}H NMR (300 MHz, DMSO): $\delta = 1.29 - 141$ (m, 10H, H-2′,3′,4′,5′,6′), 1.57 (t, 2H, $J = 6.4$ Hz, H-6), 2.46 (s, 2H, H-8), 2.75 (t, 2H, $J = 6.1$ Hz, H-5), 6.29 (bs, 2H, NH_2), 13.86 (bs, 1H, NH). – ^{13}C NMR (75 MHz, DMSO): $\delta = 21.2$ (C-3′,5′), 21.6 (C-5), 26.1 (C-4′), 31.8 (C-6), 32.8 (C_q), 34.8 (C-8), 35.3 (C-2′,6′), 114.6 (C-4b), 127.9 (C-4a), 129.1 (C-8a), 147.4 (C-9a), 152.8 (C=O), 166.3 (C=S). – HRMS (ESI): $m/z = 322.1023$ (calcd. 322.1048 for $C_{15}H_{20}N_3OS_2$, $[M+H]^+$).

*3-(Ethoxycarbonyl)amino-2-thioxo-4-oxo-1,2,3,4,5,8-hexahydro-spirobenzo[b]thieno[2,3-d]-pyrimidine-7(6H),1′-cyclohexane (**4b**)*

The product was obtained, following the general procedure, as colorless crystals from methanol (0.61 g, 79 %); m. p. 132 – 133 °C. – ^{1}H NMR (300 MHz, DMSO): $\delta = 1.26$ (t, 3H, $J = 7.1$ Hz, $COOCH_2CH_3$), 1.32 – 143 (m, 10H, H-2′,3′,4′,5′,6′), 1.59 (t, 2H, $J = 6.4$ Hz, H-6), 2.49 (s, 2H, H-8), 2.72 (t, 2H, $J = 6.1$ Hz, H-5), 4.14 (q, 2H, $J = 7.1$ Hz, $COOCH_2CH_3$), 10.16 (bs, 1H, NH), 13.71 (bs, 1H, NH). – ^{13}C NMR (75 MHz, DMSO): $\delta = 14.5$ (CH_3), 21.2 (C-3′,5′), 21.6 (C-5), 26.1 (C-4′), 31.7 (C-6), 32.5 (C_q), 35.2 (C-8), 35.5 (C-2′,6′), 61.1 (CH_2, ester), 115.3 (C-4b), 128.4 (C-4a), 129.9 (C-8a), 147.4 (C-9a), 152.8 (C=O), 166.3 (C=S). – HRMS (ESI): $m/z = 394.1230$ (calcd. 394.1259 for $C_{18}H_{24}N_3O_3S_2$, $[M+H]^+$).

*3-(2-Ethoxy-2-oxoethyl)-2-thioxo-4-oxo-1,2,3,4,5,8-hexahydro-spirobenzo[b]thieno[2,3-d]-pyrimidine-7(6H),1′-cyclohexane (**4c**)*

The product was obtained, following the general procedure, as colorless crystals from methanol (0.63 g, 81 %); m. p. 189 – 190 °C. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.25$ (t, 3H, $J = 7.1$ Hz, $COOCH_2CH_3$), 1.29 – 137 (m, 10H, H-2′,3′,4′,5′,6′), 1.57 (t, 2H, $J = 6.2$ Hz, H-6), 2.39 (s, 2H, H-8), 2.78 (t, 2H, $J = 6.2$ Hz, H-5), 4.22 (q, 2H, $J = 7.1$ Hz, $COOCH_2CH_3$), 5.17 (s, 2H, N-CH_2), 11.95 (bs, 1H, NH). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.2$ (CH_3), 21.8 (C-3′,5′), 22.0 (C-5), 26.5 (C-4′), 32.1 (C-6), 33.1 (C_q), 35.8, 35.9 (C-2′,6′), 36.1 (C-8), 47.3 (N-CH_2), 61.9 (CH_2, ester), 116.5 (C-4b), 128.8 (C-4a), 131.2 (C-8a), 148.9 (C-9a), 154.4 (C=O), 154.9 (C=O, ester), 174.6 (C=S). – HRMS (ESI): $m/z = 393.1333$ (calcd. 393.1307 for $C_{19}H_{25}N_2O_3S_2$, $[M+H]^+$).

*Synthesis of 3-(2-ethoxy-2-oxoethyl)-2-propargylsulfanyl-4-oxo-3,4,5,8-tetrahydro-spiro-benzo[b]thieno[2,3-d]-pyrimidine-7(6H),1′-cyclohexane (**5**)*

Propargyl bromide (1.10 g, 9 mmol) was added to a suspension of compound **4c** (1.18 g, 3 mmol) and *i*Pr_2NEt (0.70 g, 6 mmol) in DMF (15 mL) and the resulting mixture was stirred at room temperature for 6 h. The solution was evaporated to dryness *in vacuo*. The residue was diluted with water and then extracted with CH_2Cl_2 (3×30 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and the solvents evaporated under reduced pressure. The obtained product was purified by column chromatography. Yield: 0.91 g (71 %); m. p. 112 – 113 °C. – $R_f = 0.65$ (CH_2Cl_2, 100 %). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.24$ (t, 3H, $J = 7.1$ Hz, $COOCH_2CH_3$), 1.31 – 140 (m, 10H, H-2′,3′,4′,5′,6′), 1.60 (t, 2H, $J = 6.2$ Hz, H-6), 2.18 (bs, 1H, C-CH), 2.50 (s, 2H, H-8), 2.86 (t, 2H, $J = 5.8$ Hz, H-5), 3.96 (d, 2H, $J = 2.4$ Hz, CH_2-C), 4.20 (q, 2H, $J = 7.1$ Hz, $COOCH_2CH_3$), 4.76 (s, 2H, N-CH_2). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$ (CH_3), 21.3, 21.8 (C-3′,5′), 22.2 (C-5), 26.6 (C-4′), 32.5 (C-6), 32.9 (C_q), 36.0 (C-2′,6′), 36.3 (C-8), 44.5 (N-CH_2), 54.2 (CH_2-C), 62.1 (CH_2, ester), 72.2 (C-*CH*), 77.7 (*C*-CH), 118.5 (C-4a), 130.3 (C-8a), 131.7 (C-4b), 153.7 (C-9a), 157.9 (C=O), 162.0 (C=O, ester), 166.8 (C-2). – HRMS (ESI): $m/z = 431.1476$ (calcd. 431.1463 for $C_{22}H_{27}N_2O_3S_2$, $[M+H]^+$).

*Synthesis of **6a – c***

The azido compound (1.0 mmol) was added to the alkyne substrate **5** (1.0 mmol) in CH_3OH-H_2O (1:1) (25 mL). Then sodium ascorbate (0.4 mmol) and $CuSO_4 \cdot 5H_2O$ (0.2 mmol) were added. The mixture was stirred at room temperature for the specified time. The mixture was concentrated, diluted with H_2O and extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. Column chromatography purification gave the products **6a**–**c**.

*3-(2-Ethoxy-2-oxoethyl)-2-[(1-(2,3,4,6-tetra-o-acetyl-β-d-glucopyranosyl)-1H-1,2,3-triazol-4-yl)methyl]sulfanyl-4-oxo-3,4,5,8-tetrahydro-spirobenzo[b]thieno[2,3-d]-pyrimidine-7(6H),1′-cyclohexane (**6a**)*

The product was obtained, following the general procedure under stirring at r. t. for 16 h as colorless crystals (0.73 g, 91 %); m. p. 180 – 181 °C. – $R_f = 0.34$ (CH_2Cl_2-CH_3OH, 98:2). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.22$ (t, 3H, $J = 7.1$ Hz, $COOCH_2CH_3$), 1.33 – 144 (m, 10H, H-2′,3′,4′,5′,6′), 1.62 (t, 2H, $J = 6.4$ Hz, H-6), 1.76 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 2.52 (s, 2H, H-8), 2.88 (t, 2H, $J = 6.1$ Hz, H-5), 3.90 – 4.21 (m, 5H, $COOCH_2CH_3$, C_5H-CH_2-OCO, C_5H-CH_2-OCO), 4.47 – 4.53 (br, 2H, CH_2-$C_{triazole}$), 4.76 (s, 2H, N-CH_2), 5.18 (m, 1H, C_3H), 5.33 (m, 2H, C_2H, C_4H), 5.76 (d, 1H, $J = 6.4$ Hz, C_1H-$N_{triazole}$), 7.78 (s, 1H, $CH_{ar-triazole}$). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$ (CH_3), 20.1, 20.5, 20.6, 20.7 (4CO*CH*$_3$), 21.8 (C-3′,5′), 22.2 (C-5), 26.6 (C-4′), 27.1 (CH_2-C), 32.4 (C-6), 32.9 (C_q), 36.0 (C-2′,6′), 36.4 (C-8), 44.5 (N-CH_2), 61.5 (C_5H-CH_2-OCO), 62.0 (CH_2, ester), 67.7 (C_4H), 70.2 (C_2H), 72.6 (C_3H), 75.2 (C_5H), 85.9 (C_1H-$N_{triazole}$), 118.6 (C-4a), 121.6 ($CH_{ar-triazole}$), 130.5 (C-8a), 131.5 (C-4b), 142.2 ($C_{q-triazole}$), 154.6 (C-9a), 158.0 (C=O), 162.0 (C=O, ester), 166.9 (C-2), 168.7, 169.4, 169.9, 170.5 (4*CO*CH$_3$). – HRMS (ESI): $m/z = 804.2420$ (calcd. 804.2584 for $C_{36}H_{46}N_5O_{12}S_2$, $[M+H]^+$).

*3-(2-Ethoxy-2-oxoethyl)-2-[(1-(2,3,4,6-tetra-o-acetyl-β-d-galactopyranosyl)-1H-1,2,3-triazol-4-yl)methyl]sulfanyl-4-oxo-3,4,5,8-tetrahydro-spirobenzo[b]thieno[2,3-d]-pyrimidine-7(6H),1′-cyclohexane (**6b**)*

The product was obtained, following the general procedure, under stirring at r. t. for 12 h as colorless crystals (0.75 g, 94 %); m. p. 92 – 93 °C. – $R_f = 0.39$ (CH_2Cl_2-CH_3OH, 98:2). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.23$ (t, 3H, $J = 7.1$ Hz, $COOCH_2CH_3$), 1.33 – 144 (m, 10H, H-2′,3′,4′,5′,6′), 1.62 (t, 2H, $J = 6.3$ Hz, H-6), 1.78 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.50 (s, 2H, H-8), 2.88 (t, 2H, $J = 5.9$ Hz, H-5), 4.19 (m, 5H, $COOCH_2CH_3$, C_5H-CH_2-OCO, C_5H-CH_2-OCO), 4.44 – 4.58 (m, 2H, CH_2-$C_{triazole}$), 4.76 (s, 2H, N-CH_2), 5.17 (dd, 1H, $J_1 = 3.3$ Hz, $J_2 = 10.3$ Hz, C_3H), 5.47 (m, 2H, C_2H, C_4H), 5.73 (d, 1H, $J = 9.2$ Hz, C_1H-$N_{triazole}$), 7.79 (s, 1H, $CH_{ar-triazole}$). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$ (CH_3), 20.2, 20.5, 20.6, 20.7 (4CO*CH*$_3$), 21.8 (C-3′,5′), 22.2 (C-5), 26.6 (C-4′), 27.2 (CH_2-C),

32.5 (C-6), 32.9 (C_q), 36.0 (C-2′,6′), 36.1 (C-8), 44.5 (N-CH_2), 61.2 (C_5H-CH_2-OCO), 62.0 (CH_2, ester), 66.8 (C_4H), 67.8 (C_2H), 70.8 (C_3H), 74.0 (C_5H), 86.3 (C_1H-$N_{triazole}$), 118.6 (C-4a), 121.7 ($CH_{ar-triazole}$), 130.5 (C-8a), 131.4 (C-4b), 143.9 ($C_{q-triazole}$), 157.7 (C-9a), 158.0 (C=O), 162.0 (C=O, ester), 166.9 (C-2), 168.9, 169.8, 169.9, 170.3 (4COCH$_3$). – HRMS (ESI): $m/z = 804.2360$ (calcd. 804.2584 for $C_{36}H_{46}N_5O_{12}S_2$, $[M+H]^+$.

*3-(2-Ethoxy-2-oxoethyl)-2-[(1-(octadecyl)-1H-1,2,3-triazol-4-yl)methyl]sulfanyl-4-oxo-3,4,5,8-tetrahydro-spirobenzo-[b]thieno[2,3-d]pyrimidine-7(6H),1′-cyclohexane (**6c**)*

The product was obtained, following the general procedure, under stirring at r. t. for 18 h as colorless crystals (0.66 g, 90 %); m. p. 62 – 63 °C. – $R_f = 0.46$ (CH_2Cl_2-CH_3OH, 98:2). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.82$ (t, 3H, $J = 7.0$ Hz, CH_3), 1.20 (m, 35H, 16$CH_{2-octadecyl}$, COOCH$_2$$CH_3$), 1.33 – 144 (m, 10H, H-2′,3′,4′,5′,6′), 1.61 (t, 2H, $J = 6.4$ Hz, H-6), 1.82 (s, 2H, CH_2-N), 2.51 (s, 2H, H-8), 2.88 (t, 2H, $J = 5.9$ Hz, H-5), 4.18 (q, 2H, $J = 7.0$ Hz, COOCH_2CH$_3$,), 4.26 (bs, 2H, CH_2-$C_{triazole}$), 4.78 (bs, 2H, N-CH$_2$), 7.73 (bs, 1H, $CH_{ar-triazole}$). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$ (2CH_3), 21.8 (C-3′,5′), 22.2 (C-5), 22.7 (CH_2CH$_3$), 26.6 (C-4′), 26.7 (CH_2-C), 28.9, 29.4, 29.5, 29.6, 29.7, 30,1, 31.9 (15 CH_2), 32.5 (C-6), 32.9 (C_q), 36.0 (C-2′,6′), 36.4 (C-8), 44.5 (N-CH_2), 51.5 (CH_2-N), 62.0 (CH_2, ester), 118.5 (C-4a), 120.8 ($CH_{ar-triazole}$), 130.5 (C-8a), 131.3 (C-4b), 146.8 ($C_{q-triazole}$), 154.9 (C-9a), 158.0 (C=O), 162.0 (C=O, ester), 166.9 (C-2). – HRMS (ESI): $m/z = 726.4340$ (calcd. 726.4451 for $C_{40}H_{64}N_5O_3S_2$, $[M+H]^+$).

*Synthesis of compound **8***

A solution of sodium ethoxide in ethanol (0.3 equiv.) was added to a stirred solution of the glycoside tetraacetate **6a** (0.81 g, 1 mmol, 1.0 equiv.) in EtOH (10 mL) at r. t. The mixture was stirred for 3 h (TLC showed complete conversion). The precipitate that formed was filtered off, washed with ethanol and crystallized from DMF-water to give compound **8** as colorless crystals. Yield 54 %; m. p. 268 – 270 °C. – ^{1}H NMR (300 MHz, DMSO): $\delta = 1.31$ (t, 6H, $J = 6.4$ Hz, 2COOCH$_2$$CH_3$), 1.33 – 144 (m, 20H, 2H-2′,3′,4′,5′,6′), 1.60 (t, 4H, $J = 6.2$ Hz, 2H-6), 2.50 (s, 4H, 2H-8), 2.78 (bs, 4H, 2H-5), 4.36 (q, 4H, $J = 6.7$ Hz, 2COOCH_2CH$_3$), 4.45 (s, 4H, 2N-CH$_2$). – ^{13}C NMR (75 MHz, DMSO): $\delta = 13.9$ (CH_3), 21.3 (C-3′,5′), 21.9 (C-5), 26.2 (C-4′), 32.1 (C-6), 32.5 (C_q), 35.3 (C-2′,6′), 35.5 (C-8), 42.9 (N-CH_2), 64.6 (CH_2, ester), 116.3 (C-4b), 127.7 (C-4a), 129.2 (C-8a), 153.3 (C-9a), 157.5 (C=O), 161.3 (C-2), 169.4 (C=O, ester). – HRMS (ESI): $m/z = 391.1233$ (calcd. 391.1150 for $C_{38}H_{46}N_4O_6S_4$, $[M]^{2+}$).

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