Carbohydrate Research 461 (2018) 32–37

Contents lists available at ScienceDirect

Carbohydrate Research

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

Synthesis of bidesmosidic lupane saponins – comparison of batch and continuous-flow methodologies

Anna Korda, Zbigniew Pakulski*, Piotr Cmoch, Katarzyna Gwardiak, Romuald Karczewski

Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

ARTICLE INFO

Article history:
Received 28 February 2018
Accepted 13 March 2018
Available online 15 March 2018

Keywords:
Lupane bidesmosides
Glycosylation
Betulin
The Wagner-Meerwein rearrangement
Microreactors

ABSTRACT

Synthesis of lupane bidesmosides was optimized. The title compounds were obtained by glycosylation of 3-*O*- or 28-*O*-substituted betulin monodesmosides with Schmidt donors catalyzed by TMSOTf. Classical batch procedure and microreactor technique were used and compared in the above synthesis. Experimental results clearly showed that both methods are comparable, although any particular outcome strongly depends on the structure of the reagents. Undesired allobetulin derivatives formed by the Wagner-Meerwein rearrangement were usually isolated in minute amounts. In the case of batch reaction, shorter reaction time significantly decreased formation of side-products.

© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Betulin and betulinic acid – pentacyclic triterpenoids belonging to the lupane family and easily accessible from bark of *Betula* species – are regarded as interesting bioactive natural compounds and valuable starting materials in synthesis [1,2]. Nevertheless, their pharmacological use is often limited due to the poor solubility in water. These problems can be addressed by introduction of polar moieties at the C-3 and/or C-28 positions, e.g. by glycosylation to form saponins. Saponins, owing to their broad range of medicinal and biological properties, attract attention of numerous research groups and their chemical synthesis has been recently reviewed [3–6]. Monodesmosidic saponins of betulin were synthesized and their structure-activity relationship studies were reported [7–10]. In contrast, the betulin bidesmosides persist as a difficult to prepare target (Fig. 1). When the hydroxyl group at the C-28 position in lupane skeleton is targeted during glycosylation process, the Wagner-Meerwein rearrangement (Scheme 1) leading to allobetulin derivatives usually predominates [11–15], although some exceptions are known [16,17]. Since the bidesmosidic saponins are considered to be less haemolytic than monodesmosidic congeners, it is worthwhile to develop a general and efficient method of their synthesis [18,19].

Glycosylation reaction, although universally performed for over a century, continues to be a synthetic challenge due to the fact that numerous factors affect the outcome of the process (stereoelectronic effects, conformation and stereochemistry of reagents, concentration, temperature, reaction time, to name a few). Recently, flow reactors became available in laboratory environment and they can offer advantage over traditional batch reactors, due to the differences in reagent contact time, mixing characteristics and heat transport [20,21]. In the recent example of oligosaccharide synthesis, flow reactor proved to be superior over traditional flask methodology because better control of the contact of the reagents minimized side reaction [22–28].

As stated earlier, synthesis of lupane saponins by glycosylation of the 28-OH group of betulin derivatives **1** is often accompanied by the Wagner-Meerwein rearrangement, promoted by the Lewis acids, leading to allobetulin derivatives **2** (Scheme 1) [29]. In the case of betulin glycosylated at the 28-*O*-position (**3**), the recently observed sugar migration induced by the Wagner-Meerwein rearrangement leading to 3-*O*-glycosylated allobetulin **4** derivatives must also be considered as potentially high-yielding side-reaction (Scheme 2) [30].

Herein, we report on the synthesis of bidesmosidic betulin saponins by the glycosylation of monodesmosides and side-by-side comparison of the batch and flow methodologies. Two approaches to the bidesmosides were proposed, differing in order of attaching sugar moieties to the betulin core. In the first approach, monodesmoside **5** [30] substituted at the 28-*O* position was glycosylated at the 3-OH position in reaction with the trichloroacetimidate sugar donors under Schmidt's procedure [31,32].

* Corresponding author.
E-mail address: zbigniew.pakulski@icho.edu.pl (Z. Pakulski).

https://doi.org/10.1016/j.carres.2018.03.006
0008-6215/© 2018 Elsevier Ltd. All rights reserved.

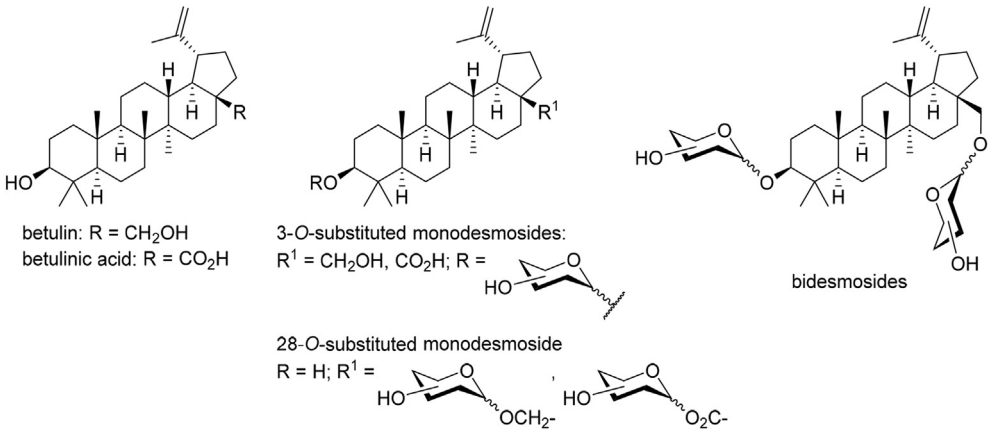
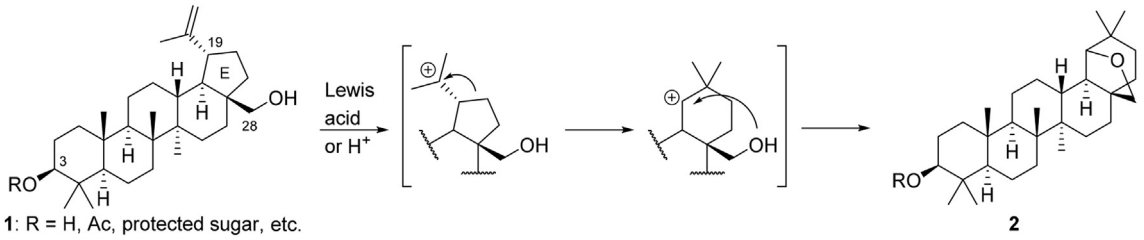
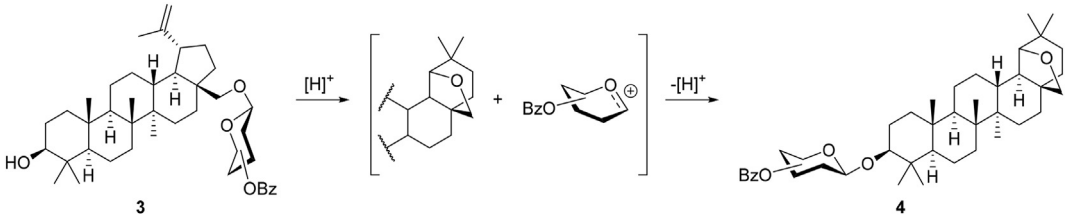


Fig. 1. Lupane type triterpenoids and saponins.



Scheme 1. The Wagner-Meerwein rearrangement of betulin derivatives.



Scheme 2. Sugar migration induced by the Wagner-Meerwein rearrangement.

In the second approach, compound **16** [11] substituted at the 3-*O* position was used as a starting material. Classical flask technique and microflow methodology were compared to identify the best conditions for the preparation of lupane bidesmosides.

2. Results and discussion

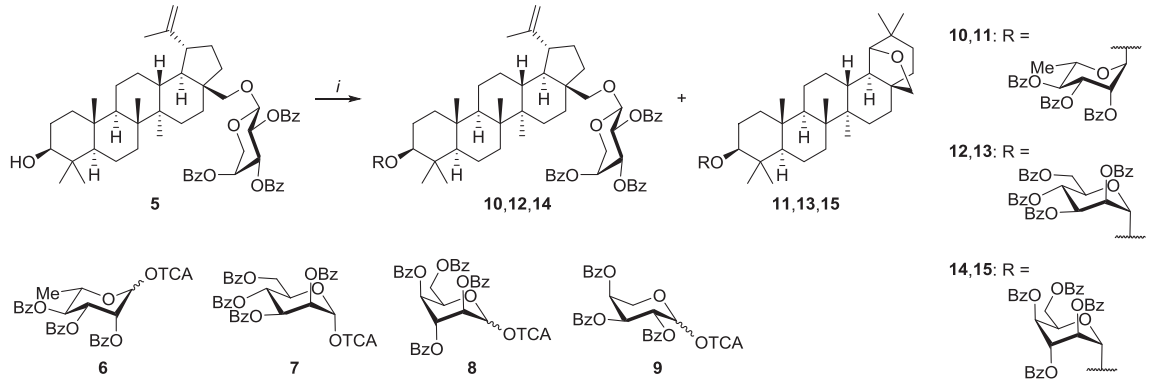
In the initial batch experiment, monodesmosidic saponin **5** glycosylated at the 28-*O* position was reacted with L-rhamnopyranosyl trichloroacetimidate **6** [33] under standard conditions (Scheme 3) [34]. Expected bidesmoside **10** [17] was obtained in 67% yield together with allobetulin glycoside **11** [30] which was isolated in 28% yield (Table 1, entry 1). Shorter reaction time inhibited formation of the rearranged product **11**, but at the cost of lower yield of desired product **10** (Table 1, entry 2, 46%).

Next, the microreactor was used to carry out the same reaction (Fig. 2). Flow experiments were performed in a coil reactor (PTFE microtube, OD × ID × L, 1/16" × 0.040" × 2 m) equipped with two Y-connectors and immersed in a cooling bath as described in previous paper [27]. Preliminary experiments allowed to determine the optimal residence time and concentration that resulted in a full conversion of the starting materials. The highest yield of bidesmoside **10** was observed for reaction performed at −40 °C (87%, Table 1, entry 4). In most cases, allobetulin glycoside **11** was also isolated in low to moderate yield (11–31%) as a by-product.

Reaction of **5** with D-mannopyranosyl trichloroacetimidate **7** [35] performed at −40 °C was selective and afforded required bidesmoside **12** as the only product (Table 1, entries 8, 10, and 12). Under flow conditions, even at 0 °C, rearrangement rate was low and allobetulinyl mannoside **13** [30] was obtained in 6–14% yield, only (Table 1, entries 9, 11). Under optimal conditions bidesmoside **12** was isolated in 78–80% yield (Table 1, entries 7 and 9).

Similarly, glycosylation of **5** with D-idopyranosyl trichloroacetimidate **8** [16] gave bidesmoside **14** in high yield (86–88%; Table 1, entries 13, 16, and 18). Allobetulinyl idopyranoside **15**, i.e. a by-product, was observed at a low yield (5–23%).

In the second step, monodesmosidic saponin **16**, with sugar moiety at the 3-*O* position was used as a starting material (Scheme 4, Table 2). Its reaction with L-rhamnopyranosyl donor **6** at −40 °C was highly selective towards bidesmosidic saponin **17** which was isolated in approx. 83% from batch experiments (Table 2, entries 1, 2), and in 95% from microflow reaction (Table 2, entry 4). At 0 °C moderate amount (18–20%) of allobetulin **21** [30] was also obtained (Table 2, entries 3, 5). Reaction with D-manno derivative **7**



Scheme 3. *i*: Glycosyl donor (**6**-**8**), CH_2Cl_2, TMSOTf.

Table 1
Synthesis of bidesmosidic saponins by glycosylation of monodesmoside **5**.

Entry	Glycosyl donor	Reaction mode	Time (min)	Temp. (°C)	TMSOTf	Bidesmosidic Saponin (isolated yield, %)	Allobetulin glycoside (isolated yield, %)
1	**6**	batch	30	−40	60 mol%	**10** (67)	**11** (28)
2	**6**	batch	5	−40	60 mol%	**10** (46)	–
3	**6**	flow	4	0	0.01 M	**10** (49)	**11** (31)
4	**6**	flow	4	−40	0.01 M	**10** (87)	**11** (11)
5	**6**	flow	4	0	0.003 M	**10** (56)	**11** (12)
6	**6**	flow	4	−40	0.003 M	**10** (57)	–
7	**7**	batch	30	−40	60 mol%	**12** (78)	**13** (16)
8	**7**	batch	5	−40	60 mol%	**12** (57)	–
9	**7**	flow	4	0	0.01 M	**12** (80)	**13** (14)
10	**7**	flow	4	−40	0.01 M	**12** (51)	–
11	**7**	flow	4	0	0.003 M	**12** (56)	**13** (6)
12	**7**	flow	4	−40	0.003 M	**12** (53)	–
13	**8**	batch	30	−40	60 mol%	**14** (86)	**15** (8)
14	**8**	batch	5	−40	60 mol%	**14** (73)	–
15	**8**	flow	4	0	0.01 M	**14** (50)	**15** (23)
16	**8**	flow	4	−40	0.01 M	**14** (87)	**15** (12)
17	**8**	flow	4	0	0.003 M	**14** (37)	**15** (5)
18	**8**	flow	4	−40	0.003 M	**14** (88)	–

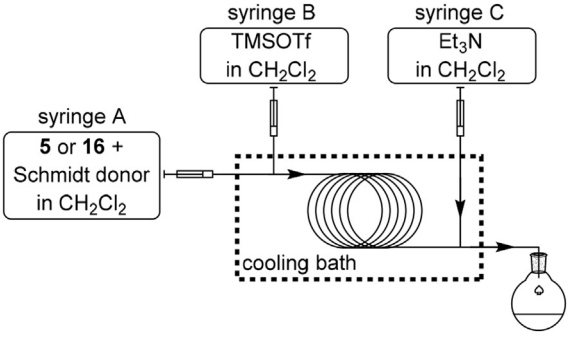
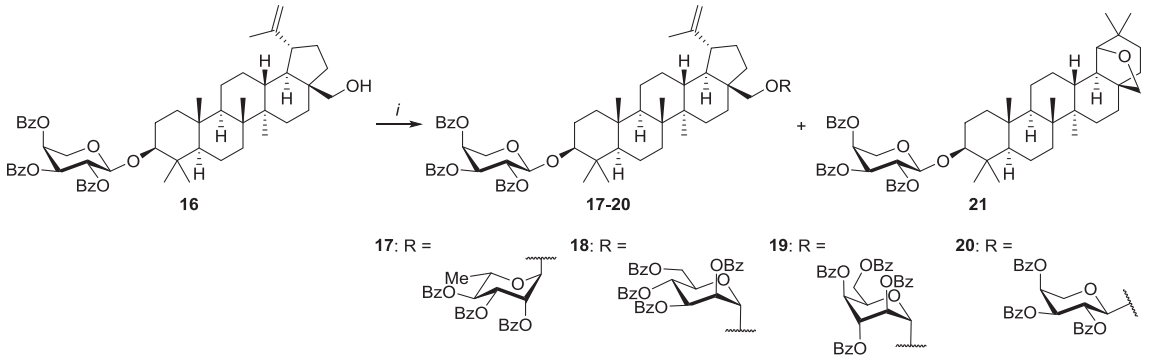


Fig. 2. Coil microreactor.

was, however, less selective. Disubstituted product **18** was isolated in 54% yield under batch technique (Table 2, entry 8), and in 79% yield under flow conditions; allobetulin **21** was isolated in 7% (Table 2, entry 10).

The highest yield of D-idopyranoside **19** was observed under classical conditions (93%, Table 2, entry 13); minute amounts of allobetulinyl derivative **21** (5%) were also isolated. Both yield and selectivity were lower in microreactor, reaching 72% yield of the expected bidesmoside **19** at −40 °C (Table 2, entry 16). Similarly, bis-L-arabinoside **20** was prepared by glycosylation of **16** with the known L-arabinopyranosyl trichloroacetimidate **9** [36]. The highest yield was obtained in batch experiments (79–81%, Table 2, entries 19 and 20), whereas in microflow reaction the isolated yield and selectivity were slightly lower (71% in best run, Table 2, entry 23).

As expected, the stereoselectivity of glycoside bond formation was governed by the presence of benzoyl protecting groups in sugar donors [37]. In all cases, the 1,2-*trans* linkage was formed exclusively as confirmed by the chemical shifts of anomeric protons and carbon atoms as well as $J_{1,2}$ coupling constants. Observed rearrangement of L-arabinopyranoside **16** to the allobetulin glycoside **21** was expected. It is well known that due to the presence of free 28-OH group such compounds are prone to the Wagner-Merweein rearrangement. On the other hand, the loss of the arabinopyranosyl fragment from the 28-*O* position and rearrangement to allobetulin during glycosylation of saponin **5** leading to glycosides **11**, **13**, and **15** was observed for the first time. Recently we described a similar transformation where the sugar migration from the 28-*O* to 3-*O* position was forced by the Wagner-Meerwein rearrangement [30]. It required, however, a much longer reaction time, higher temperature and relatively high concentration of the Lewis acid. Here, the loss of a sugar moiety was complete within minutes. Interestingly, migration of the arabinopyranosyl fragment from the 28-*O* to 3-*O* position was not observed. It may suggest that glycosylation of acceptor **5** in the 3-*O* position by external Schmidt donor is much faster than migration of sugar fragment from 28-*O* position.



Scheme 4. *i*: Glycosyl donor (**6**-**9**), CH_2Cl_2, TMSOTf.

Table 2
Synthesis of bidesmosidic saponins by glycosylation of monodesmoside **16**.

Entry	Glycosyl donor	Reaction mode	Time (min)	Temp. (°C)	TMSOTf	Bidesmosidic saponin (isolated yield, %)	Allobetulin glycoside 21 (isolated yield, %)
1	**6**	batch	30	−40	60 mol%	**17** (83)	–
2	**6**	batch	5	−40	60 mol%	**17** (82)	–
3	**6**	flow	4	0	0.01 M	**17** (71)	(20)
4	**6**	flow	4	−40	0.01 M	**17** (95)	–
5	**6**	flow	4	0	0.003 M	**17** (61)	(18)
6	**6**	flow	4	−40	0.003 M	**17** (67)	–
7	**7**	batch	30	−40	60 mol%	**18** (47)	(15)
8	**7**	batch	5	−40	60 mol%	**18** (54)	–
9	**7**	flow	4	0	0.01 M	**18** (39)	(20)
10	**7**	flow	4	−40	0.01 M	**18** (79)	(7)
11	**7**	flow	4	0	0.003 M	**18** (57)	(13)
12	**7**	flow	4	−40	0.003 M	**18** (53)	(4)
13	**8**	batch	30	−40	60 mol%	**19** (93)	(5)
14	**8**	batch	5	−40	60 mol%	**19** (40)	–
15	**8**	flow	4	0	0.01 M	**19** (52)	(37)
16	**8**	flow	4	−40	0.01 M	**19** (72)	(10)
17	**8**	flow	4	0	0.003 M	**19** (43)	(18)
18	**8**	flow	4	−40	0.003 M	**19** (63)	–
19	**9**	batch	30	−40	60 mol%	**20** (81)	(19)
20	**9**	batch	5	−40	60 mol%	**20** (79)	–
21	**9**	flow	4	0	0.01 M	**20** (63)	(20)
22	**9**	flow	4	−40	0.01 M	**20** (56)	–
23	**9**	flow	4	0	0.003 M	**20** (71)	(20)
24	**9**	flow	4	−40	0.003 M	**20** (54)	–

3. Conclusions

Herein we described the first synthesis of betulin bidesmosides in a microfluidic system and compared the flow methodology with the classical batch technique. A series of saponins were synthesized by a glycosylation of monodesmosides with Schmidt donors. Presented results demonstrate that the synthesis of betulin bidesmosides can be efficiently realized using either the classical batch or the flow methodologies, but a very careful control of the reaction conditions is necessary to limit formation of undesired allobetulin derivatives. The target bidesmosides were isolated in a high yield. In some cases, the easy to separate products of the Wagner-Meerwein rearrangement were also isolated as by-products. Usually, the higher yield and/or better selectivity toward bidesmosides were observed for the reactions carried out in microreactor. In batch experiments, the shorter reaction time restricted formation of allobetulin derivatives.

Presented methodologies may be considered as highly effective synthetic methods for the preparation of lupane bidesmosides. However, according to our experience, susceptibility of lupane monodesmosides to the Wagner-Meerwein rearrangement is somewhat unpredictable. The exact results depend on the structure of the starting materials and products, thus optimization of the reaction conditions is highly advisable in every case. In our opinion, development of a general method for lupane bidesmosides synthesis is being hindered by unusual chemical properties of betulin and betulin saponins. The above problems may be perfectly concluded with the famous Paulsen's observation, that "*each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know-how. There are no universal reaction conditions for oligosaccharide syntheses*" [38].

4. Experimental

General procedures. Silica gel HF_{254} was used for TLC and preparative TLC. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR spectra were recorded at 298 K with a Varian NMR-vnmrs600 or vnmrs500 spectrometers, using standard experimental conditions and Varian software (ChemPack 4.1). Configurational assignments were based on the NMR measurements, generated using two-dimensional techniques like COSY

and ^{1}H-^{13}C gradient selected HSQC (*g*-HSQC), as well as ^{1}H-^{13}C gradient selected HMBC (*g*-HMBC). Internal TMS was used as the ^{1}H and ^{13}C NMR chemical shift standard. *J* values are given in Hertz. High-resolution mass spectra (HRMS ESI) were acquired with Mariner and MaldiSYNAPT G2-S HDMS (Waters) mass spectrometers. Optical rotations were recorded on a Jasco P-2000 automatic polarimeter.

4.1. Glycosylation of saponins **5** and **16**

Method A – batch methodology. A solution of glycosyl donor (**6**-**9**, 0.10 mmol) and acceptor **5** or **16** (71 mg, 0.08 mmol) in dichloromethane (8 mL) was stirred for 20 min at room temperature over molecular sieves (4 Å, 300 mg, finely ground), then cooled to −40 °C, TMSOTf (10 μL, 0.06 mmol) was added, and the mixture was stirred (see Tables for details), quenched with Et_3N (0.5 mL), and the solvents were removed under diminished pressure. Column chromatography (hexane–ethyl acetate, 40: 1 → 5: 1) of the residue yielded the protected saponins as white foam.

Method B – microflow technique. Syringe A (Fig. 2) was filled with solution of a glycosyl donor (0.10 mmol) and the corresponding acceptor (0.08 mmol) in dry dichloromethane (5 mL). Syringe B contained solution of TMSOTf (0.01 M or 0.003 M in dichloromethane, 6 mL). The reagents were then fed into the microreactor filled with dichloromethane via a double syringe pump and the residence time was adjusted to 4 min by controlling the pumping rate. Reaction was quenched by injection of 5% solution of triethylamine in dichloromethane placed in syringe C into the reaction stream at the bath temperature. Reaction mixture leaving microreactor was collected, concentrated and the residue was purified by column chromatography as described in *Method A*.

4.1.1. 28-O-2′,3′,4′-Tri-O-benzoyl-α-L-arabinopyranosylbetulin 3β-O-2″,3″,4″,6″-tetra-O-benzoyl-α-D-mannopyranoside (**12**)

$[\alpha]_D^{20}$ 64.2 (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.27–8.10 (m, 35 H, H_{Ar}), 6.09 (t, 1 H, $J_{4,3}=J_{4,5}=10.1$ Hz, H-4″), 5.91 (dd, 1 H, $J_{3,2}$ 3.2, $J_{3,4}$ 10.1 Hz, H-3″), 5.73 (dd, 1 H, $J_{2,1}$ 6.2, $J_{2,3}$ 8.6 Hz, H-2′), 5.68–5.70 (m, 1 H, H-4′), 5.61–5.64 (m, 2 H, H-2″, H-3′), 5.27 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1″), 4.71 (d, 1 H, $J_{1,2}$ 6.2 Hz, H-1′), 4.65–4.69 (m, 2 H, H-6″, H-29), 4.52–4.56 (m, 2 H, H-5″, H-29), 4.47 (dd, 1 H, $J_{6,5}$ 5.0, $J_{6,6'}$ 12.0 Hz, H-6″), 4.33 (dd, 1 H, $J_{5,4}$ 4.1, $J_{5,5'}$ 12.7 Hz, H-5′), 3.92 (dd, 1 H, $J_{5,4}$ 1.9, $J_{5,5'}$ 12.7 Hz, H-5′), 3.68 (d, 1 H, *J* 8.8 Hz, H-28), 3.59 (d, 1 H, *J* 8.8 Hz, H-28), 3.33 (dd, 1 H, *J* 4.3, 11.7 Hz, H-3), 2.34–2.39 (m, 1 H), 1.95–2.02 (m, 2 H), 1.79–1.85 (m, 2 H), 0.63–1.70 (m, 20 H, lupane protons), 1.66 (s, 3 H, CH_3), 1.10 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3), 0.87 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.8, 165.6, 165.6, 165.5, 165.1, 150.4 (C-20), 128.3–133.4 (C_{Ar}), 109.7 (C-29), 101.7 (C-1′), 94.3 (C-1″), 84.2 (C-3), 71.6, 70.5, 70.2, 70.0, 69.4, 68.6 (C-28), 68.5, 67.0, 63.1 (C-6″), 62.6 (C-5′), 55.6 (C-5), 50.3, 48.7, 47.9, 47.0 (C), 42.6 (C), 40.8 (C), 38.6 (C), 38.2 (CH_2), 37.6, 37.1 (C), 34.8 (CH_2), 33.9 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 28.8, 27.0 (CH_2), 25.1 (CH_2), 22.2 (CH_2), 20.8 (CH_2), 19.0, 18.2 (CH_2), 16.5, 16.1, 15.9, 14.8. HR-MS (ESI) calc. for $C_{90}H_{96}NaO_{18}$ $[M+Na]^+$: 1487.6494. Found: 1487.6495.

4.1.2. 28-O-2′,3′,4′-Tri-O-benzoyl-α-L-arabinopyranosylbetulin 3β-O-2″,3″,4″,6″-tetra-O-benzoyl-α-D-idopyranoside (**14**)

$[\alpha]_D^{20}$ 79.6 (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.14–8.17 (m, 35 H, H_{Ar}), 5.73 (dd, 1 H, $J_{2,1}$ 6.3, $J_{2,3}$ 8.6 Hz, H-2′), 5.68–5.69 (m, 1 H, H-4′), 5.63 (dd, 1 H, $J_{3,2}$ 8.6, $J_{3,4}$ 3.5 Hz, H-3′), 5.58 (br s, 1 H, H-3″ or H-4″), 5.41 (br s, 1 H, H-3″ or H-4″), 5.32 (br s, 1 H, H-1″), 5.19 (br s, 1 H, H-2″), 4.97–4.99 (m, 1 H, H-5″), 4.70 (d, 1 H, $J_{1,2}$ 6.3 Hz, H-1′), 4.65–4.68 (m, 2 H, H-6″, H-29), 4.56–4.60 (m, 2 H, H-6″, H-29), 4.33 (dd, 1 H, $J_{5,4}$ 4.1, $J_{5,5'}$ 12.8 Hz, H-5′), 3.91 (dd, 1 H, $J_{5,4}$ 1.9, $J_{5,5'}$ 12.8 Hz, H-5′), 3.68 (d, 1 H, *J* 8.8 Hz, H-28), 3.58 (d, 1 H, *J* 8.8 Hz, H-28), 3.34 (dd, 1 H, *J* 4.3, 11.7 Hz, H-3), 2.34–2.38 (m, 1 H, H-19), 1.96–2.01 (m, 2 H, H-21, H-22), 1.82–1.85 (m, 2 H, H-1, H-2), 1.66 (s, 3 H, H-30), 1.62 (H-12), 1.57 (H-13), 1.49 (H-18), 1.45 (H-2), 1.39 (H-15), 1.38 (H-6), 1.37 (H-21), 1.35 (H-11), 1.28 (H-6), 1.24 (H-16), 1.14 (H-7, H-11), 1.12 (H-9), 1.04 (H-7, H-16), 1.02 (s, 3 H, H-23), 1.00 (H-22), 0.99 (H-12), 0.88 (s, 3 H, H-26), 0.87 (s, 3 H, H-27), 0.76 (s, 3 H, H-25), 0.70 (s, 3 H, H-24), 0.70 (H-1), 0.64 (H-15), 0.52 (H-5). ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.7, 165.6, 165.3, 165.2, 165.1, 164.7, 150.3 (C-20), 128.2–133.5 (C_{Ar}), 109.7 (C-29), 101.6 (C-1′), 93.7 (C-1″), 82.9 (C-3), 70.5 (C-3′), 70.0 (C-2′), 68.6 (C-28), 68.4 (C-4′), 68.1 (C-2″), 67.2 (C-3″ or C-4″), 66.8 (C-3″ or C-4″), 64.6 (C-5″), 63.6 (C-6″), 62.5 (C-5′), 55.8 (C-5), 50.3 (C-9), 48.7 (C-18), 47.9 (C-19), 47.0 (C-17), 42.6 (C-14), 40.7 (C-8), 38.4 (C-4), 38.2 (C-1), 37.6 (C-13), 37.0 (C-10), 34.8 (C-22), 33.8 (C-7), 29.7 (C-21), 29.2 (C-16), 28.5 (C-23), 27.0 (C-15), 25.1 (C-12), 21.5 (C-2), 20.8 (C-11), 19.0 (C-30), 18.1 (C-6), 16.2 (C-24), 16.0 (C-25), 15.8 (C-26), 14.9 (C-27). HR-MS (ESI) calc. for $C_{90}H_{96}NaO_{18}$ $[M+Na]^+$: 1487.6494. Found: 1487.6497.

4.1.3. 3β-O-(2,3,4,6-Tetra-O-benzoyl-α-D-idopyranosyl) allobetulin (**15**)

$[\alpha]_D^{20}$ 70.5 (*c* 0.2, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.15–8.17 (m, 20 H, H_{Ar}), 5.59 (br s, 1 H), 5.41 (br s, 1 H), 5.32 (br s, 1 H) 5.20 (br s, 1 H), 4.97–5.00 (m, 1 H, H-5′), 4.68 (dd, 1 H, $J_{6,5}$ 8.2, $J_{6,6'}$ 11.5 Hz, H-6′), 4.59 (dd, 1 H, $J_{6,5}$ 4.3, $J_{6,6'}$ 11.5 Hz, H-6′), 3.78 (d, 1 H, *J* 7.6, H-28), 3.53 (s, 1 H, H-19), 3.45 (d, 1 H, *J* 7.6, H-28), 3.36 (dd, 1 H, *J* 4.3, 11.7 Hz, H-3), 0.56–1.88 (m, 24 H, lupane protons), 1.02 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3), 0.81 (s, 3 H, CH_3), 0.80 (s, 3 H, CH_3), 0.70 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.3, 165.2, 164.7, 133.5, 133.2, 128.2–130.2 (C_{Ar}), 93.9 (C-1′), 87.9 (C-19), 83.1 (C-3), 71.2 (CH_2), 68.2, 67.2, 66.8, 64.6, 63.7 (CH_2), 56.0, 51.0, 46.8, 41.5 (C), 40.7 (C), 40.6 (C), 38.4 (CH_2), 37.1 (C), 36.8 (CH_2), 36.3 (C), 34.1, 33.9 (CH_2), 32.7 (CH_2), 28.8, 28.5, 26.4 (CH_2), 26.3 (CH_2), 24.5, 21.6 (CH_2), 21.0 (CH_2), 18.2 (CH_2), 16.4, 16.2, 15.7, 13.6. HR-MS (ESI) calc. for $C_{64}H_{76}NaO_{11}$ $[M+Na]^+$: 1043.5285. Found: 1043.5283.

4.1.4. 3β-O-2′,3′,4′-Tri-O-benzoyl-α-L-arabinopyranosylbetulin 28-O-2″,3″,4″-tri-O-benzoyl-α-L-rhamnopyranoside (**17**)

$[\alpha]_D^{20}$ 107.0 (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.23–8.11 (m, 30 H, H_{Ar}), 5.79 (dd, 1 H, $J_{3,2}$ 3.4, $J_{3,4}$ 10.0 Hz, H-3″), 5.76 (dd, 1 H, $J_{2,1}$ 6.4, $J_{2,3}$ 8.9 Hz, H-2′), 5.69 (dd, 1 H, $J_{2,1}$ 1.6, $J_{2,3}$ 3.4 Hz, H-2″), 5.67–5.68 (m, 1 H, H-4′), 5.66 (t, 1 H, $J_{4,3}=J_{4,5}=10.0$ Hz, H-4″), 5.59 (dd, 1 H, $J_{3,2}$ 8.9, $J_{3,4}$ 3.6 Hz, H-3′), 4.96 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1″), 4.78 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1′), 4.73 (br s, 1 H, H-29), 4.61 (br s, 1 H, H-29), 4.33 (dd, 1 H, $J_{5,4}$ 3.9, $J_{5,5'}$ 12.9 Hz, H-5′), 4.14 (dq, 1 H, $J_{5,4}$ 10.0, $J_{5,6}$ 6.3 Hz, H-5″), 3.87 (dd, 1 H, $J_{5,4}$ 2.0, $J_{5,5'}$ 12.9 Hz, H-5′), 3.60 (d, 1 H, *J* 9.3 Hz, H-28), 3.56 (d, 1 H, *J* 9.3 Hz, H-28), 3.14 (dd, 1 H, *J* 4.7, 11.7 Hz, H-3), 2.47–2.51 (m, 1 H), 2.04–2.09 (m, 2 H), 1.96–2.02 (m, 1 H), 1.83–1.86 (m, 1 H), 0.63–1.81 (m, 20 H, lupane protons), 1.72 (s, 3 H, CH_3), 1.38 (d, 3 H, *J* 6.3 Hz, H-6″), 0.99 (s, 3 H, CH_3), 0.98 (s, 3 H, CH_3), 0.80 (s, 3 H, CH_3), 0.77 (s, 3 H, CH_3), 0.64 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.8, 165.6, 165.6, 165.5, 165.2, 150.4 (C-20), 128.2–133.4 (C_{Ar}), 109.7 (C-29), 103.0 (C-1′), 98.2 (C-1″), 90.1 (C-3), 71.8 (C-4″), 70.8 (C-2″), 70.7 (C-3′), 70.2 (C-2′, C-3″), 68.7 (C-4′), 66.9 (C-5″, C-28), 62.6 (C-5′), 55.5 (C-5), 50.3, 48.8, 47.9, 47.1 (C), 42.7 (C), 40.9 (C), 39.0 (C), 38.7 (CH_2), 37.6, 36.8 (C), 35.1 (CH_2), 34.1 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 27.7, 27.1 (CH_2), 26.1 (CH_2), 25.2 (CH_2), 20.8 (CH_2), 19.2, 18.1 (CH_2), 17.8 (C-6″), 16.0, 14.7. HR-MS (ESI) calc. for $C_{83}H_{92}NaO_{16}$ $[M+Na]^+$: 1367.6283. Found: 1367.6289.

4.1.5. 3β-O-2′,3′,4′-Tri-O-benzoyl-α-L-arabinopyranosylbetulin 28-O-2″,3″,4″,6″-tetra-O-benzoyl-α-D-mannopyranoside (**18**)

$[\alpha]_D^{20}$ 37.3 (*c* 0.2, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.25–8.11 (m, 35 H, H_{Ar}), 6.13 (t, 1 H, $J_{4,3}=J_{4,5}=10.1$ Hz, H-4″), 5.88 (dd, 1 H, $J_{3,2}$ 3.3, $J_{3,4}$ 10.1 Hz, H-3″), 5.75 (dd, 1 H, $J_{2,1}$ 6.4, $J_{2,3}$ 8.8 Hz, H-2′),

5.72–5.74 (dd, 1 H, $J_{2,1}$ 1.4, $J_{2,3}$ 3.3 Hz, H-2″), 5.66–5.68 (m, 1 H, H-4′), 5.59 (dd, 1 H, $J_{3,2}$ 8.8, $J_{3,4}$ 3.6 Hz, H-3′), 5.06 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1″), 4.78 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1′), 4.69–4.71 (m, 2 H, H-6″, H-29), 4.60 (br s, 1 H, H-29), 4.48 (dd, 1 H, $J_{6,5}$ 3.8, $J_{6,6'}$ 12.2 Hz, H-6″), 4.31–4.38 (m, 2 H, H-5′, H-5″), 4.04 (d, 1 H, J 9.2 Hz, H-28), 3.87 (dd, 1 H, $J_{5,4}$ 1.8, $J_{5,5'}$ 12.8 Hz, H-5′), 3.25 (d, 1 H, J 9.2 Hz, H-28), 3.13 (dd, 1 H, J 4.6, 11.6 Hz, H-3), 2.38–2.43 (m, 1 H), 1.98–2.11 (m, 3 H), 0.63–1.85 (m, 25 H, lupane protons), 1.70 (s, 3 H, CH_3), 0.98 (s, 3 H, CH_3), 0.93 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 0.75 (s, 3 H, CH_3), 0.62 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.8, 165.6, 165.5, 165.5, 165.4, 165.2, 150.2 (C-20), 128.3–133.4 (C_{Ar}), 109.8 (C-29), 103.0 (C-1′), 98.5 (C-1″), 90.1 (C-3), 70.7, 70.5, 70.2, 69.2, 68.6, 67.5 (C-28), 66.9, 62.8 (C-5′), 55.5 (C-5), 50.3, 48.8, 48.0, 47.0 (C), 42.7 (C), 40.8 (C), 39.0 (C), 38.8 (CH_2), 37.6, 36.8 (C), 34.6 (CH_2), 34.2 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 27.7, 27.2 (CH_2), 26.1 (CH_2), 25.2 (CH_2), 20.8 (CH_2), 18.1 (CH_2), 16.0, 14.8. HR-MS (ESI) calc. for $C_{90}H_{96}NaO_{18}$ $[M+Na]^+$: 1487.6494. Found: 1487.6482.

*4.1.6. 3β-O-2′,3′,4′-Tri-O-benzoyl-α-L-arabinopyranosylbetulin 28-O-2″,3″,4″,6″-tetra-O-benzoyl-α-D-idopyranoside (**19**)*

$[\alpha]_D^{20}$ 82.8 (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.15–8.15 (m, 35 H, H_{Ar}), 5.75 (dd, 1 H, $J_{2,1}$ 6.5, $J_{2,3}$ 8.8 Hz, H-2′), 5.66–5.67 (m, 1 H, H-4′), 5.62 (br s, 1 H, H-3″ or H-4″), 5.59 (dd, 1 H, $J_{3,2}$ 8.8, $J_{3,4}$ 3.6 Hz, H-3′), 5.39 (br s, 1 H, H-3″ or H-4″), 5.25 (br s, 1 H, H-2″), 5.09 (br s, 1 H, H-1″), 4.82–4.85 (m, 1 H, H-5″), 4.78 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1′), 4.68 (br s, 1 H, H-29), 4.65 (dd, 1 H, $J_{6,5}$ 7.3, $J_{6,6'}$ 11.5 Hz, H-6″), 4.61 (dd, 1 H, $J_{6,5}$ 6.1, $J_{6,6'}$ 11.5 Hz, H-6″), 4.58 (br s, 1 H, H-29), 4.32 (dd, 1 H, $J_{5,4}$ 3.8, $J_{5,5'}$ 12.8 Hz, H-5′), 4.05 (d, 1 H, J 9.2 Hz, H-28), 3.87 (dd, 1 H, $J_{5,4}$ 1.8, $J_{5,5'}$ 12.8 Hz, H-5′), 3.25 (d, 1 H, J 9.2 Hz, H-28), 3.12 (dd, 1 H, J 4.6, 11.5 Hz, H-3), 2.32–2.36 (m, 1 H, H-19), 0.59–2.02 (m, 25 H, lupane protons), 1.66 (s, 3 H, H-30), 0.92 (s, 3 H, CH_3), 0.83 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 0.70 (s, 3 H, CH_3), 0.62 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.0, 165.8, 165.6, 165.3, 165.2, 165.1, 164.5, 150.2 (C-20), 128.2–133.5 (C_{Ar}), 109.8 (C-29), 103.0 (C-1′, $^1J(^{13}C\text{-}^1H)$: 159.8 Hz), 98.6 (C-1″, $^1J(^{13}C\text{-}^1H)$: 170.8 Hz), 90.1 (C-3), 70.7 (C-3′), 70.2 (C-2′), 68.6 (C-4′), 67.3 (C-2″), 67.2 (C-28), 66.8 (C-3″ or C-4″), 66.5 (C-3″ or C-4″), 64.4 (C-5″), 62.9 (C-6″), 62.5 (C-5′), 55.5 (C-5), 50.3, 48.8, 48.0, 47.0, 42.6, 40.7, 39.0, 38.6, 37.5, 36.8, 34.5, 34.0, 29.7, 27.7, 27.1, 26.0, 25.0, 20.6, 18.0, 16.0, 16.0, 15.9, 147. HR-MS (ESI) calc. for $C_{90}H_{96}NaO_{18}$ $[M+Na]^+$: 1487.6494. Found: 1487.6488.

*4.1.7. 3β-O-2′,3′,4′-Tri-O-benzoyl-α-L-arabinopyranosylbetulin 28-O-2″,3″,4″-tri-O-benzoyl-α-L-arabinopyranoside (**20**)*

$[\alpha]_D^{20}$ 131.2 (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.30–8.07 (m, 30 H, H_{Ar}), 5.75 (dd, 1 H, $J_{2,1}$ 6.4, $J_{2,3}$ 8.8 Hz, H-2′), 5.72 (dd, 1 H, $J_{2,1}$ 6.3, $J_{2,3}$ 8.6 Hz, H-2″), 5.66–5.69 (m, 2 H, H-4′, H-4″), 5.58–5.62 (m, 2 H, H-3′, H-3″), 4.77 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1′), 4.68 (d, 1 H, $J_{1,2}$ 6.3 Hz, H-1″), 4.65 (br s, 1 H, H-29), 4.55 (br s, 1 H, H-29), 4.30–4.34 (m, 2 H, H-5′, H-5″), 3.90 (dd, 1 H, $J_{6,5}$ 1.9, $J_{6,6'}$ 12.8 Hz, H-5′ or H-5″), 3.87 (dd, 1 H, $J_{5,4}$ 1.8, $J_{5,5'}$ 12.9 Hz, H-5′ or H-5″), 3.66 (d, 1 H, J 8.9 Hz, H-28), 3.56 (d, 1 H, J 8.9 Hz, H-28), 3.12 (dd, 1 H, J 4.7, 11.6 Hz, H-3), 2.32–2.37 (m, 1 H), 1.93–2.00 (m, 2 H), 1.75–1.85 (m, 3 H), 0.95–1.62 (m, 18 H, lupane protons), 1.64 (s, 3 H, CH_3), 0.86 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 0.78 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 0.65 (s, 3 H, CH_3), 0.58–0.60 (m, 1 H, H-5). ^{13}C NMR ($CDCl_3$) δ: 165.8, 165.7, 165.6, 165.2, 165.1, 150.4 (C-20), 128.2–133.5 (C_{Ar}), 109.6 (C-29), 103.0 (C-1′ or C-1″), 101.7 (C-1′ or C-1″), 90.1 (C-3), 70.7, 70.5, 70.2, 70.0, 68.7 (C-28), 68.5, 62.6 (C-5′, C-5″), 55.5 (C-5), 50.3, 48.7, 47.9, 47.0 (C), 42.5 (C), 40.7 (C), 39.0 (C), 38.7 (CH_2), 37.6, 36.8 (C), 34.8 (CH_2), 33.8 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 27.7, 27.0 (CH_2), 26.1 (CH_2), 25.1 (CH_2), 20.8 (CH_2), 19.0, 18.0 (CH_2), 16.1, 15.8, 14.7. HR-MS (ESI) calc. for $C_{82}H_{90}NaO_{16}$ $[M+Na]^+$: 1353.6127. Found: 1353.6143.

Acknowledgment

This work was financially supported by the National Science Centre, Poland (No. 2016/21/B/ST5/02141).

Appendix A. Supplementary data

Supporting Information Available. Copies of ^{1}H and ^{13}C NMR spectra of all new compounds are provided.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.carres.2018.03.006.

References

[1] E.W.H. Hayek, U. Jordis, W. Moche, F. Sauter, Phytochemistry 28 (1989) 2229–2242.
[2] P.A. Krasutsky, Nat. Prod. Rep. 23 (2006) 919–942.
[3] Y. Yang, S. Laval, B. Yu, Adv. Carbohydr. Chem. Biochem. 71 (2014) 137–226.
[4] L. Zu, Y. Zhao, G. Gu, J. Carbohydr. Chem. 33 (2014) 269–297.
[5] C. Gauthier, J. Legault, M. Pichon-Gauthier, A. Pichette, Phytochemistry Rev. 10 (2011) 521–544.
[6] C. Gauthier, J. Legault, A. Pichette, Mini-Reviews Org. Chem. 6 (2009) 321–344.
[7] D. Thibeault, C. Gauthier, J. Legault, J. Bouchard, P. Dufour, A. Pichette, Bioorg. Med. Chem. 15 (2007) 6144–6157.
[8] P. Cmoch, A. Korda, L. Rárová, J. Oklešťková, M. Strnad, R. Luboradzki, Z. Pakulski, Tetrahedron 70 (2014) 2717–2730.
[9] K. Sidoryk, A. Korda, L. Rárová, J. Oklešťková, M. Strnad, P. Cmoch, Z. Pakulski, K. Gwardiak, R. Karczewski, R. Luboradzki, Tetrahedron 71 (2015) 2004–2012.
[10] I. Podolak, A. Galanty, D. Sobolewska, Phytochemistry Rev. 9 (2010) 425–474.
[11] C. Gauthier, J. Legault, S. Lavoie, S. Rondeau, S. Tremblay, A. Pichette, J. Nat. Prod. 72 (2009) 72–81.
[12] C. Gauthier, J. Legault, S. Lavoie, S. Rondeau, S. Tremblay, A. Pichette, Tetrahedron 64 (2008) 7386–7399.
[13] L.E. Odinokova, G.I. Oshitok, V.A. Denisenko, V.F. Anufriev, A.M. Tolkach, N.I. Uvarova, Chem. Nat. Prod. 20 (1984) 168–173.
[14] N.I. Uvarova, L.N. Atopkina, G.B. Elyakov, Carbohydr. Res. 83 (1980) 33–42.
[15] N.I. Uvarova, G.I. Oshitok, G.B. Elyakov, Carbohydr. Res. 27 (1973) 79–87.
[16] P. Cmoch, A. Korda, L. Rárová, J. Oklešťková, M. Strnad, K. Gwardiak, R. Karczewski, Z. Pakulski, Eur. J. Org Chem. (2014) 4089–4098.
[17] Y. Li, J. Sun, B. Yu, Org. Lett. 13 (2011) 5508–5511.
[18] K. Oda, H. Matsuda, T. Murakami, T. Ohgitani, M. Yoshikawa, Biol. Chem. 381 (2000) 67–74.
[19] L. Voitquenne, C. Lavaud, G. Massiot, L. LeMen-Olivier, Pharm. Biol. 40 (2002) 253–262.
[20] R.L. Hartman, J.P. McMullen, K.F. Jensen, Angew. Chem. Int. Ed. 50 (2011) 7502–7519 (and references cited therein).
[21] J. Wegner, S. Ceylan, A. Kirschning, Adv. Synth. Catal. 354 (2012) 17–57.
[22] D.M. Ratner, E.R. Murphy, M. Jhunjhunwala, D.A. Snyder, K.F. Jensen, P.H. Seeberger, Chem. Commun. (2005) 578–580.
[23] F.R. Carrel, K. Geyer, J.D.C. Codée, P.H. Seeberger, Org. Lett. 9 (2007) 2285–2288.
[24] K. Geyer, T. Gustafsson, P.H. Seeberger, Synlett (2009) 2382–2391.
[25] D.T. McQuade, P.H. Seeberger, J. Org. Chem. 78 (2013) 6384–6389.
[26] S. Mostarda, P. Filipponi, R. Sardella, F. Venturoni, B. Natalini, R. Pellicciaria, A. Gioiello, Org. Biomol. Chem. 12 (2014) 9592–9600.
[27] Z. Pakulski, P. Cmoch, Tetrahedron 71 (2015) 4757–4769.
[28] S. Matthies, D.T. McQuade, P.H. Seeberger, Org. Lett. 17 (2015) 3670–3673.
[29] For the review on allobetulin and its derivatives see W. Dehaen, A.A. Mashentseva, T.S. Seitemboetov, Molecules 16 (2011) 2443–2466.
[30] A. Korda, Z. Pakulski, P. Cmoch, K. Gwardiak, R. Karczewski, Tetrahedron 73 (2017) 1740–1744.
[31] R.R. Schmidt, J. Michel, Angew. Chem., Int. Ed. Engl. 19 (1980) 731–732.
[32] X. Zhu, R.R. Schmidt, Angew. Chem., Int. Ed. Engl. 48 (2009) 1900–1934.
[33] C. Gauthier, J. Legault, M. Lebrun, P. Dufour, A. Pichette, Bioorg. Med. Chem. 14 (2006) 6713–6725.
[34] K. Kuczynska, P. Cmoch, L. Rárová, J. Oklešťková, A. Korda, Z. Pakulski, M. Strnad, Carbohydr. Res. 423 (2016) 49–69.
[35] B. Becker, R.H. Furneaux, F. Reck, O.A. Zubkov, Carbohydr. Res. 315 (1999) 148–158.
[36] B. Yu, J. Xie, S. Deng, Y. Hui, J. Am. Chem. Soc. 121 (1999) 12196–12197.
[37] P. Fugedi, in: D.E. Levy, P. Fugedi (Eds.), The Organic Chemistry of Sugars, CRC Press, Boca Raton, 2006 (Chapter 4).
[38] H. Paulsen, Angew. Chem. Int. Ed. 21 (1982) 155–173.