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Electrosynthesis of Stable Betulin-Derived Nitrile Oxides and their Application in Synthesis of Cytostatic Lupane-Type Triterpenoid-Isoxazole Conjugates

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Novel lupane-type triterpenoid-isoxazole conjugates were designed by direct placing of isoxazole linker at C(17) of triterpenoid. The suggested synthetic sequence demonstrates successful combination of electro-organic synthesis and conventional approaches. TEMPO-mediated electrooxidation of betulin to betulinal was developed and optimized at boron-doped diamond anodes with potassium acetate as inexpensive supporting electrolyte. Betulinal-derived oxime was further selectively electro-oxidized at a graphite anode to nitrile oxide, which proved to be stable and isolable species. The same reaction sequence was performed with 3β-lupane-3,28-diol. Nitrile oxides were characterized by ^{15}N NMR and X-ray crystallography. The isolable nitrile oxides allowed creation of isoxazole library by 1,3-dipolar cycloaddition reactions with various alkynes. Some of the title conjugates exhibit cytostatic properties against breast cancer cell line MCF7, glioblastoma multiform cell line U-87 MG and lung carcinoma cell line A549 with growth inhibition (GI_{50}) concentrations up to 11 μM, while being harmless to immortalized human fibroblasts hTERT (GI_{50} > 100 M).

Introduction

Betulin (**1**) is a naturally occurring pentacyclic lupane-type triterpenoid (lup-20(29)-ene-3β,28-diol), which is found in wide variety of plants, but mostly in the outer bark of birch trees (*Betula sp.*, *Betulaceae*). Historically observed anticancer and antiviral activity of betulin, its abundance in the bark (up to 30% of dry weight), and a facile isolation process made it an attractive target for the synthesis of pharmacologically important semisynthetic analogues.[1] Literature reports during the past decade demonstrate broad spectrum of biological properties of modified betulin analogues that include antibacterial,[2] antimalarial,[3] anti-inflammatory,[4] anti-diabetic,[5] anti-hyperlipidemic activity,[6] and a potential to inhibit human immunodeficiency virus (HIV).[7] Since the first report by Trumbull et al. in 1976,[8] on anticancer activity of betulinic acid against lymphocytic leukemia, there have been many reports on the preparation of novel betulin derivatives with enhanced therapeutic efficiency against different cancer cell lines and improved solubility in biological media.[9] It has also been demonstrated that installation of a nitrogen-containing heterocyclic system to the triterpenoid scaffold can greatly improve their biological activity.[10] 1,2,3-Triazoles as a common example of azoles are mostly introduced using the copper(I)-catalyzed alkyne-azide cycloaddition reaction on *O*- or *N*-propargyl function installed at different positions (C(3), C(28), C(19)) of the betulin scaffold.[11] On the other hand, isoxazole moiety also belongs to the accepted linkers[12] and amide isosteres[13] in medicinal chemistry. To the best of our knowledge, there are only few reports of betulin isoxazole conjugates, in which the isoxazole ring was attached to the betulin core using modification in triterpenoid A ring (Figure 1). For example, Grishko published synthesis of C(1)–C(2) fused isoxazole-betulin derivatives **A** and their cytotoxicity studies against different tumor cell lines.[14] Also for betulin type triterpenoids containing C(2)–C(3) fused isoxazoles **B** have been studied for their anticancer activity and as hypoxia-inducible factor prolyl hydrolase and osteoclastogenesis inhibitors.[15]

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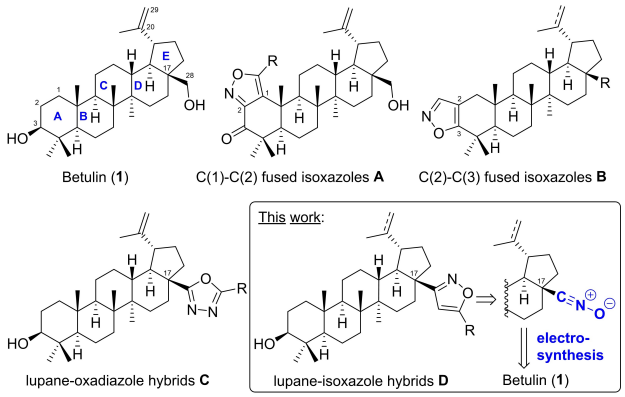


Figure 1. Diversity of betulin -isoxazole and betulin-oxadiazole hybrids.

We were interested in the installation of isoxazole moiety by forming a C–C bond at the C(17) position of betulin (lupane-type triterpenoid-isoxazole hybrids **D**, Figure 1). The only precedent in the literature for the synthesis and cytotoxic activity of C(17)-linked lupane-type triterpenoid-azole hybrids of type **C** was explored by Shults and co-workers.[16]

Isoxazoles among other methods are frequently synthesized by 1,3-dipolar cycloaddition reaction (1,3-DCR) between alkyne and nitrile oxide.[17] The latter can be obtained either by dehydration of nitromethyl moiety[18] or oxidation of an aldoxime[19] which is commonly done by electrophilic chlorination followed by HCl elimination.[20] Electrophilic C(20)–C(29) double bond of betulin skeleton is not compatible with such a classic approach.

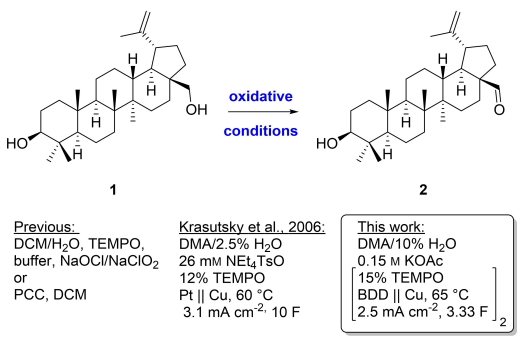
Therefore, the aldoxime oxidation route seems like an ideal platform to challenge the electro-organic synthesis. Indeed, electro-organic synthesis has begun to reemerge as an attractive and competitive technique alongside classical synthesis in the recent decades, almost 200 years after the first steps were made by Kolbe.[21–24] The avoidance of stoichiometric chemical oxidizers and reducing agents leads to a significant lowering of reagent waste. Furthermore, these often expensive and hazardous substances e.g. (hypo)chlorites or chromium(VI) reagents and other transition metal compounds, which are commonly used in classical synthesis routes, are cancelled out from the process and replaced by inexpensive electricity[21,22,24,25] or – in the case of active electrodes – can be immobilized as the system's working electrode and recycled *in situ*.[26,27] Recently, electrosynthesis was employed to generate anti-tumor agents.[28] Against the background of the increasing expansion of the renewable energy sector,[29] electricity is thus up to becoming the green reagent of the 21st century and economically attractive.[30]

Here we report electro-organic synthesis of exceptionally stable betulin-derived nitrile oxide and its further transformation into novel C(17)-linked lupane-type triterpenoid-isoxazole hybrids. The key steps of the sequence are 1) a selective electrooxidation of primary C(28)-alcohol to aldehyde; 2) an electrochemical generation of nitrile oxides from the corresponding oximes; 3) cycloaddition with various alkynes. The developed sequence demonstrates application of electrooxidation for acquiring semisynthetic betulin derivatives that are important addition to biologically active triterpenoids.

Results and Discussion

Protocols for betulin oxidation into its corresponding C(28)-aldehyde **2** are readily available in literature.[31–44] Among these there are mostly Anelli[31–36] and Jones[31,34,37–43] type oxidations employed, both coming along with several drawbacks. In particular, the Jones oxidation and related transformations suffer from the use of highly toxic/mutagenic chemicals and a poor discrimination between the primary and the secondary alcohol moieties,[31,34,37–43] whereas *N*-oxyl radical-mediated oxidation frequently employs expensive terminal oxidizers[36] and co-catalysts[32] or reagents incompatible with the betulin 20(29)-double bond (namely bleach).[31,33–35] Moreover, the excessive usage of those reagents leads to tedious workup procedures to ensure removal of reagent traces and byproducts and creates large amounts of waste. However, only few electrooxidations for the transformation **1**→**2** have been reported.[45–47] The lipophilic nature of betulin (logP > 8)[48] classifies it as a very challenging substrate for electrosynthesis – the outstanding lipophilicity and polar, ionic feature for electrolysis seem to be contradictory. The objective complications are due to the adjustment of solvent system that would ensure sufficient solubility of the starting material, a redox mediator and a supporting electrolyte. Highly polar solvents that are common for electrochemical experiments such as water or polar organic solvents are incompatible with the unique solubility properties of betulin. On the other hand, the supporting electrolytes, which should provide the conductivity during the electrooxidation process, are mostly insoluble in less polar solvents.[49]

The generally good discrimination between primary and secondary alcohols provided by TEMPO is additionally enhanced by the presence of sterically demanding 4,4-dimethyl moiety[27,50] of betulin. In 2006, TEMPO-mediated electrooxidation has been reported by the Krasutsky group (Scheme 1).[46] They suggested the use of precious platinum electrodes and relatively costly tetraethylammonium tosylate (NEt_4TsO) as



Scheme 1. Oxidation of betulin **1**. TEMPO = 2,2,6,6-tetramethylpiperidine *N*-oxyl, PCC = pyridinium chlorochromate, DMA = *N*,*N*-dimethylacetamide, BDD = boron-doped diamond.

supporting electrolyte. Later, scale-up to a 50 L pilot apparatus with a graphite anode instead of platinum was claimed, although full experimental details are not available.[45]

Here, we report an electrooxidation protocol to produce betulinic aldehyde up to 5 g scale using considerably cheaper electrode materials and supporting electrolytes. Noteworthy, boron-doped diamond (BDD) electrodes are considered as sustainable, since they are produced from methane as carbon source and avoid critical elements such as platinum group metals.[51]

Indeed, carbon-based electrode materials turned out superior to platinum in our studies. BDD electrodes outperformed platinum as anode material by 20% higher yield (qNMR) reaching 78% in the initial experiments. Boron-doped diamond is a high performance carbon electrode which is sustainably produced, metal- and maintenance-free.[51] The commercial widely available electrode material is superior in many technically relevant electro-conversions.[52] In the beginning we chose copper as cathodic material, which turned out to be the best within the electrosynthetic screening. However, copper surprisingly exceeded BDD and graphite only by little (Figure 2a), indicating a graphite||graphite system as a particularly interesting approach for future investigation.

With 10 F and 2.5 mA cm^{-2} we found electrolysis conditions similar to the already known to be the best. An increased water content of 10% (v/v) turned out to be beneficial. This might be attributed to an enhanced hydrogen evolution reaction at the counter electrode. By increasing the water content further, we observed a drop in yield due to solubility issues. Since the catalytic cycle of TEMPO requires neutral to alkaline pH,[53] we focused on potassium hydroxide and acetate as inexpensive and sustainable alternatives of NEt_4TsO to serve as supporting electrolyte. As expected, both showed a better performance than the previously used NEt_4TsO, whereby the acetate outperformed the hydroxide (Figure 2b).

This can be explained by the formation of the electrochemically inactive oxoammonium hydroxide adduct of the mediator species at pH $\geq$ 12 as well as the decomposition of the hydroxide adduct.[54] Especially at low concentrations (30 mm) of the supporting electrolyte, potassium acetate provided significant better properties than tetraethylammonium tosylate (Figure 2c).

Next, different solvents were investigated (Figure 2d). Similarly to the previously reported protocol,[46] N,N-dimethylformamide and N,N-dimethylacetamide turned out to the best solvents. DMF was slightly better than DMA at low substrate concentration, but we observed a drastic decrease of betulinal yield in DMF moving from 45 to 113 mm of betulin solution. Finally, N,N-dimethylacetamide was assigned as the best solvent, which is operational with 113 mm (50 mg mL^{-1}) concentration betulin.

As one can expect, a high mediator concentration was beneficial for the reaction. In contrast to conventional procedures, where a reagent is added and stirring can be continued until full consumption, addition and consumption of the terminal oxidant (electricity) have to take place in the same defined time span. Therefore, a well-balanced ratio between current density and the concentration of the electron accepting species, in this case the mediator, is crucial for acceptable current efficiencies.

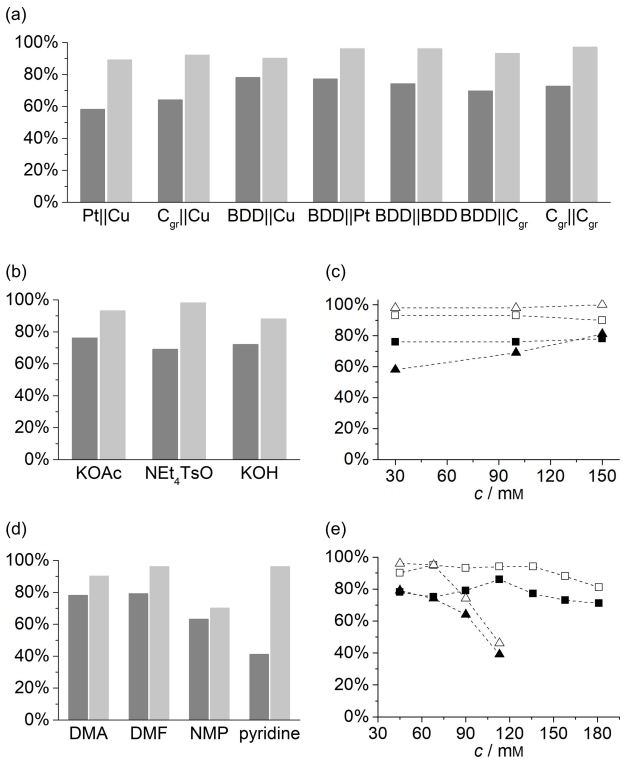


Figure 2. Parameter screening for the aldehyde **2** synthesis. (qNMR) (a) Electrode screening. 45 mM betulin, 0.15 mM KOAc, 20.5 mM TEMPO in DMA/H_2O (9:1), 65 °C, 2.5 mA cm^{-2}, 10 F. ■ yield. ■ conversion. (b) Supporting electrolyte. 45 mM betulin, 0.1 mM supporting electrolyte, 20.5 mM TEMPO in DMA/H_2O (9:1), 65 °C, BDD||Cu, 2.5 mA cm^{-2}, 10 F. ■ yield. ■ conversion. (c) Supporting electrolyte concentration. 45 mM betulin, supporting electrolyte, 20.5 mM TEMPO in DMA/H_2O (9:1), 65 °C, BDD||Cu, 2.5 mA cm^{-2}, 10 F. ■ yield (KOAc). □ conversion (KOAc). ▲ yield (Et_4NOTs). △ conversion (Et_4NOTs). (d) Solvent. 45 mM betulin, 0.15 mM KOAc, 20.5 mM TEMPO in solvent/H_2O (9:1), 65 °C, BDD||Cu, 2.5 mA cm^{-2}, 10 F. ■ yield. ■ conversion. (e) Betulin concentration. Betulin, 0.15 mM KOAc, 45% TEMPO in DMA/H_2O (9:1), 65 °C, BDD||Cu, 2.5 mA cm^{-2}, 10 F. ■ yield (DMA). □ conversion (DMA). ▲ yield (DMF). △ conversion (DMF). DMF = *N*,*N* dimethylformamide, NMP = *N* methylpyrrolidin-2-one, C_{gr} = isostatic graphite.

However, further increasing the amount of mediator above 30% did not result in a substantial amelioration in yield. Due to the solubility behavior of betulin within the electrolyte and the instability of TEMPO at elevated temperatures a maximum in yield was observed around 65 °C. This is a compromise between an acceptable amount of dissolved substrate and a high turnover number. To provide fresh, active mediator over a longer time, we thus split the electrolysis protocol in several cycles. An increased yield of 83% (+5%, qNMR) was achieved by splitting the electrolysis into three cycles with application of 3.33 F charge and addition of 15% TEMPO for each cycle. (Figure 3a) Finally, a two-cycle protocol with 3.33 F and 15% TEMPO in each cycle provided a superior yield of 89%. Further splitting of the electrolysis showed to be detrimental.

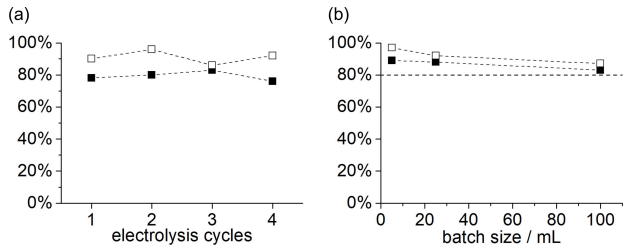


Figure 3. (a) Aldehyde **2** synthesis in dependence of number of electrolysis cycles (x). 45 mM betulin, 0.15 mM KOAc, (45/x)% TEMPO in DMA/H_2O (9:1), 65 °C, 2.5 mA cm^{-2}, BDD||Cu, (10/x) F. ■ yield (qNMR). □ conversion (qNMR). (b) Scale-up of aldehyde synthesis. 45 mM betulin, 0.15 mM KOAc in DMA/H_2O (9:1), 65 °C, two cycles à 15% TEMPO, 2.5 mA cm^{-2}, BDD||Cu, 3.33 F. ■ yield (qNMR). □ conversion (qNMR). – 113 mM betulin on 5 g/100 mL scale for comparison (isolated yield).

Scale-up of the two-cycle electrolysis to 2 g scale (100 mL vessel, Figure 3b) was possible with only a slight drop in yield. The average yield was 83% (qNMR) with a conversion of 87%, giving a yield of 95% based on the consumed starting material. To isolate the aldehyde, the crude product was precipitated by either addition of water or cooling the reaction mixture down to −50 °C. The latter may allow for a practically waste free pathway for recycling solvent and supporting electrolyte by distillation and crystallization, respectively. Following purification of the crude via column chromatography gave isolated yields of 78–80%, confirming the yields determined by qNMR. This was transferred to 5 g scale (100 mL vessel) by increasing the concentration of starting material without any loss in yield. Compared to the literature known process,[46] we were able to use a BDD anode instead of platinum and potassium acetate instead of tetraethylammonium tosylate with competitive yields. Furthermore, we present a facile workup and purification procedure, which was not done for galvanostatic electrosynthesis of betulinal before.

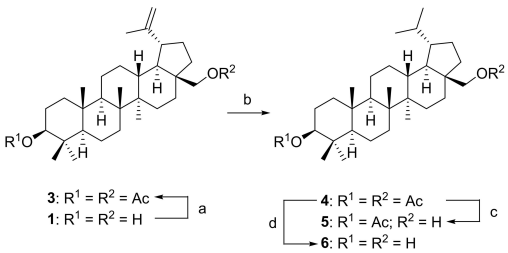
Synthesis of betulinal **2** congener lupanal **8** with a reduced C(20)–C(29) double bond started with acetylation and then hydrogenation sequence to form product **4**. We observed that acetylation drastically improves the solubility of substrate and thus facilitates the hydrogenation process.[55] Compound **4** was then fully deacetylated by KOH providing 3β,28-lupandiol **6** or selectively deacetylated with Al(O-*i*Pr)$_3$ in refluxing *i*-PrOH to form partially protected product **5** (Scheme 2).

Due to the lower solubility of compounds **5** and **6**, the electrooxidation protocol used for betulinal synthesis gave only unsatisfying yields (below 40%). However, changing to solvents providing better solubility and reducing the concentration of the starting material showed promising results. 22.5 mM lupandiol **6** in pyridine/10% H_2O with 0.15 M KOAc (BDD||Cu, 45%TEMPO, 2.5 mA cm^{-2}, 10 F) already yielded 60% of the corresponding aldehyde **7** (Scheme 3).

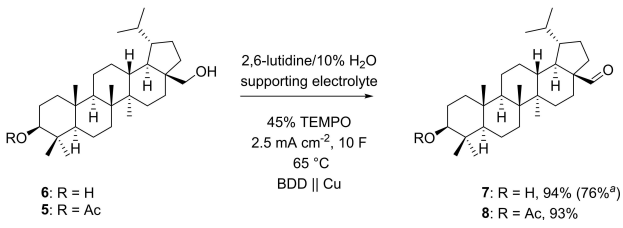
A strong discoloration of the electrolyte indicated unwanted side reactions of the solvent pyridine. To prevent this, 2,6-lutidine was chosen as a solvent exhibiting a higher stability under the electrolysis conditions, while maintaining a high solubility of triterpenoids. The poorer conductivity of 2,6-lutidine/10% H_2O/0.15 M KOAc, however, required for the addition of MTBS (16 mM) as second supporting electrolyte. Here, a yield of 92% was achieved. Significantly lower yields were achieved with the individual supporting electrolytes, MTBS and KOAc alone. Both slightly alkaline medium provided by the acetate and enhanced solubility of electrolyte conveyed by tetraalkylammonium ions is needed in the lutidine system. Consequently, tetrabutylammonium acetate (NBu_4OAc) as supporting electrolyte at a concentration of 0.1 M ensured 94% yield of aldehyde **7**, no matter if the salt was used as a solid or generated *in-situ* from acetic acid and tetrabutylammonium hydroxide solution. Scale-up of the synthesis of **7** to 250 mg scale (25 mL vessel) yielded 76%. Interestingly, no difference was observed whether one electrolysis cycle with 10 F or three cycles with 3.33 F each were done. Also, the electrooxidation of 3β-acetoxy lupane-28-ol **5** was tested and a system KOAc (0.15 mM)/MTBS (16 mM)/2,6-lutidine/10% H_2O yielded 93% of product **8**.

After the development of the electrochemical oxidation conditions for betulin **1** and compounds **5** and **6**, we continued with the synthesis of corresponding aldoximes. Condensation of aldehydes **2** and **8** (or **7**) and hydroxylamine under alkaline conditions effortlessly provided aldoximes **9** and **10** with excellent yields (Scheme 4).[41] Compound **8** was simultaneously deacetylated in the reaction medium.

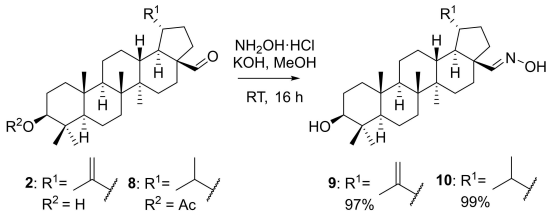
Next, we proceeded to study nitrile oxide generation and its 1,3-dipolar cycloaddition reaction. To verify the compatibility of C(20)–C(29) double bond of aldoxime **9** towards electrophilic reagents, we checked various chemical oxidation pathways and compared the reactivity of unsaturated system **9** with that of



Scheme 2. Synthesis of **5** and **6**. Reagents and conditions: (a) Ac_2O, C2 petrol, reflux, 3 h, 95%; (b) H_2, Pd/C, THF, 40 °C, 72 h, 98%; (c) Al(O-*i*Pr)$_3$, *i*-PrOH, 80 °C, 6 h, 92%; (d) KOH, EtOH, reflux, 3 h, quant.



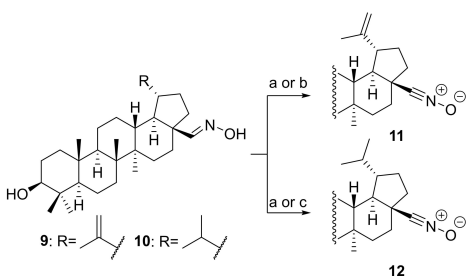
Scheme 3. Synthesis of **7** and **8**. Electrolytic conditions, 50 mg scale: boron-doped diamond (BDD) anode, Cu cathode, 10 vol.% water in 2,6-lutidine, 65 °C, j = 2.5 mA cm^{-2}, Q = 10 F, supporting electrolyte (0.1 M NBu_4OAc for **7**, 0.15 M KOAc + 16 mM MTBS for **8**), 45% TEMPO. [a] 250 mg scale. MTBS = tributylmethylammonium methylsulfate.



Scheme 4. Synthesis of aldoximes **9** and **10**. Reagents and conditions: (b) $NH_2OH \cdot HCl$, KOH, MeOH, RT, 16 h, (**9**, 97%, **10**, 99%).

saturated system **10**. We started with the well-known NCS-oxidation,[56] in which oxime **10** provided nitrile oxide **12** with 79% yield in 3 h (Scheme 5). As expected, this method was not applicable to the double-bond containing derivative **9** due to the electrophilicity of NCS. Reaction of oxime **9** with other oxidants[57] including NaOCl, chloramine-T, oxone, *m*-CPBA led predominantly to the formation of chlorinated or overoxidized byproducts, and the target product **11** was observed in amounts $<20\%$ (HPLC). Eventually, synthetically applicable result (72% yield) was achieved in the reaction of the oxime **9** with MnO_2, however, a large excess of the reagent (20 equiv.) was required, which is not compatible with scale-up[58] and the contemporary green chemistry principles. Finally, generation of double bond containing nitrile oxide **11** was achieved after 2 hours in 70% yield with (bis(trifluoroacetoxy)iodo)benzene (PIFA).[59] A slightly higher yield (79%) was obtained in the case of the saturated system - nitrile oxide **12**. The functional group tolerance and minimal required excess (1.2 equiv.) proved the advantage of PIFA in nitrile oxide synthesis compared to other reaction conditions. However, the drawback of the method is formation of iodobenzene, which on a larger scale may cause purification problems.

Nitrile oxides **11** and **12** proved to be particularly stable and their structures were proven by a high-resolution mass spectral analysis and an infrared spectrum that showed strong narrow band around 2275 cm^{-1} typical for the nitrile oxides. However, to fully prove the structure and to make a profit from their chemical stability we have also synthesized ^{15}N-labeled analogue **11′**. For this purpose, betulinic aldehyde **2** was converted into oxime **9′** using $^{15}NH_2OH \cdot HCl$, which was then subsequently



Scheme 5. Synthesis of nitrile oxides **11** and **12**. Reagents and conditions: (a) PIFA, acetone, RT, 2 h (70% of **11**, 79% of **12**); (b) MnO_2, $CHCl_3$, RT, 20 h 72%; (c) NCS, Py, CH_2Cl_2, RT, 3 h, 79%. Py = pyridine, NCS = *N*-chlorosuccinimide, PIFA = (bis(trifluoroacetoxy)iodo)benzene.

oxidized by PIFA. The ^{15}N NMR spectrum, in addition to ^{1}H and ^{13}C NMR spectra, unambiguously confirmed the formation of compounds **9′** and **11′**. The ^{15}N-labeled compounds provided chemical shifts at -25.61 ppm for the oxime **9′** and at -185.95 ppm for the nitrile oxide **11′** (Figure 4a, Figure 4b); CH_3NO_2 as the external standard at 0 ppm).[60] Analysis of ^{1}H-^{15}N ($^{2}J_{HN} = 2.2$ Hz) and ^{13}C-^{15}N ($^{1}J_{NC} = 2.2$ Hz) coupling constants revealed that oxime **9′** exists in its anti-configuration. Additionally, the molecular structure of nitrile oxide **12** was unambiguously proven by its single crystal X-ray analysis (Figure 5).[61]

The transformation of aldoximes **9** and **10** into nitrile oxides **11** and **12** seemed like a good platform to test the scope of direct electrolysis developed by Hartmer and Waldvogel in 2015[62] for the nitrile synthesis. The first efforts of aldoxime oxidation were published by Shono et al.[63] as a domino oxidation-reduction process based on platinum electrodes in combination with halogenides as mediatory system, which leads to tremendous platinum corrosion. Since nitrile oxides occur as the intermediate in the nitrile formation via domino oxidation-reduction electrolysis,[62] this pathway offers access to the nitrile oxides if the reduction is cut off. This can readily be realized by changing to a divided cell.[64] Furthermore, literature has some examples for electrosynthesis of isoxazoles and isoxazolines from oximes either by electrolysis in presence of a dipolarophile[63] or its subsequent addition to the electrolysis medium.[64] However, up to the best of our knowledge there are

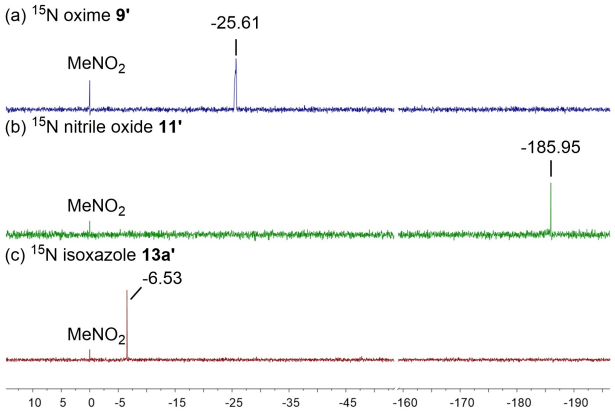


Figure 4. Comparison of ^{15}N NMR (51 MHz, $CDCl_3$) spectra for ^{15}N labeled oxime **9′**, nitrile oxide **11′** and isoxazole **13a′** using CH_3NO_2 ($\delta = 0.0$ ppm) as external standard.

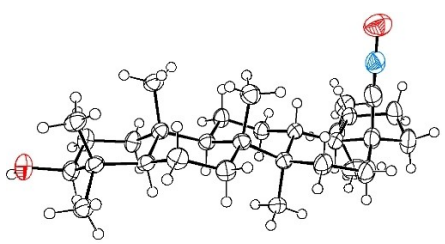


Figure 5. ORTEP representation of nitrile oxide **12**.

no examples for an electrosynthetic access to the triterpenoid-derived nitrile oxide **11** or its isoxazole congeners.

Inexpensive stainless steel was used as cathode, since it showed only poor nitrile formation and other decomposition products in the previous studies.[62] To suppress any nitrile formation, all reactions were done in semi-divided setup using stainless steel wire commercially available at a home depot. In order to ensure application of a sufficient amount of charge, 4 F were chosen as starting point. At first, different anode materials were tested. As expected, graphite showed the best performance. It is important to note that a sandpaper-polished graphite gave approximately 10% higher yields as the intercalated electrolyte constituents from the previous oxidation cycles present in the swollen electrode surface were mechanically removed.[65] Other carbon-based materials were not efficient for the conversion of betulin aldoxime and all further experiments were conducted with freshly sanded graphite anodes. In general, the nature of the carbon electrode can have significant influence onto the reproducibility of electro-conversions.[66] Next, applied charge and current density were investigated. It appeared, that corresponding to the nitrile formation an applied charge of 2.5 F is the optimal condition. However, in our case the yield increased with decreasing current density, indicating 1 mA cm^{-2} as the most practical setting (Figure 6b). Consequently, rolled carbon felt (rCF), a porous electrode material, was tested in order to further decrease the current density at the electrode surface while maintaining the same geometrical current density. Yet, it showed inferior results (Figure 6a). Screening various solvents led to alcohols, namely methanol, as optimal medium for this conversion. Interestingly, acetonitrile, the solvent of choice for the nitrile formation, showed only poor performance (Figure 6c), which seems to be owed to the lower solubility of the oxime. In addition, upon longer standing the formation of side product was observed, which by HPLC-MS was identified as a product of 1,3-DCR between the formed nitrile oxide and acetonitrile.

Since the unwanted nitrile formation must be avoided, another counter reaction must be offered to the system. Thus, water was added to the electrolyte. Similarly to the electrooxidation of betulin, water contents higher than 10% (v/v) were detrimental due to precipitation of the oxime. On the other hand, a lower water content was also insufficient for high yield nitrile oxide synthesis (Figure 6d). Subsequently, different supporting electrolytes were tested, revealing rather hydrophilic anions to be beneficial. However, highly hydrophilic anions like perchlorate showed to be disadvantageous (Figure 6e). The influence of the cation was logically comparably small, but still we observed better results for bulky lipophilic cations. Finally, the best results were achieved with 32 mM of MTBS. Investigation of the temperature dependence unveiled 50 °C to be an optimum. Lower temperature decreases solubility of oxime, but its increase leads to the decomposition of nitrile oxide. The effect of the limited solubility of the oxime was further confirmed by screening the impact of the substrate concentration. In general, a lower concentration resulted in a better yield.

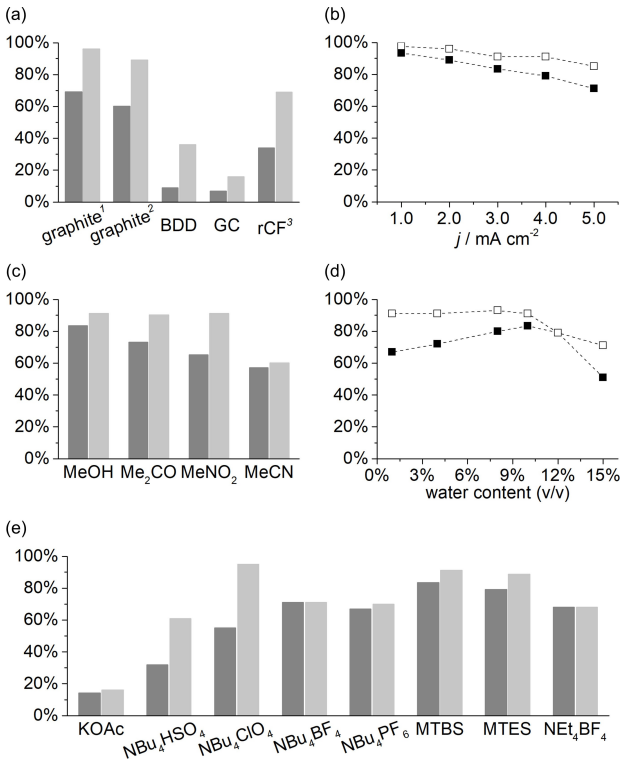
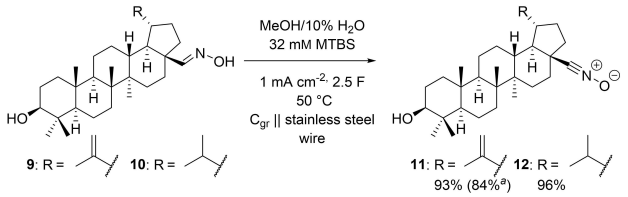


Figure 6. Parameter screening for the nitrile oxide 11 synthesis (qNMR). (a) Anode materials. 22 mM oxime, 32 mM MTBS in MeOH/H_2O (9:1), 50 °C, Anode||V2A-wire, 3 mA cm^{-2}, 4 F. [1] sanded electrode,[2] electrode only wiped, [3] 22 mM oxime, 32 mM MTBS in MeOH/H_2O (9:1), 50 °C, C_{gr}||V2A-wire, 1 mA cm^{-2}, 2.5 F. ■ yield. ■ conversion. (b) Current density. ■ yield. □ conversion. 22 mM oxime, 32 mM MTBS in MeOH/H_2O (9:1), 50 °C, C_{gr}||V2A-wire, *j*, 2.5 F. (c) Solvents. 22 mM oxime, 32 mM MTBS in solvent/H_2O (9:1), 50 °C, C_{gr}||V2A-wire, 1 mA cm^{-2}, 2.5 F. ■ yield. ■ conversion. (d) Water content. ■ yield. □ conversion.22 mM oxime, 32 mM MTBS in MeOH/H_2O, 50 °C, C_{gr}||stainless steel wire, 1 mA cm^{-2}, 2.5 F. (e) Influence of different supporting electrolytes on the nitrile oxide formation (qNMR). 22 mM oxime, 32 mM supporting electrolyte in MeOH/H_2O (9:1), 50 °C, C_{gr}||stainless steel wire, 1 mA cm^{-2}, 2.5 F. ■ yield. ■ conversion. GC = glassy carbon, rCF = rolled carbon felt, MTES = triethylmethylammonium methylsulfate.

Electrooxidation of oxime **9** at a 250 mg scale (25 mL vessel) with the optimal parameters was performed with an excellent 88% yield of product **11**, which outperforms the classical method presented above. In addition, solvent and supporting electrolyte can be easily recycled, making the electro-organic synthesis of betulin nitrile oxide **11** particularly economic and environmentally benign. Transferring the reaction into bigger vessels (50 mL, 100 mL) resulted in a yield drop due to the longer electrolysis time, in particular the prolonged heating of already formed nitrile oxide. However, with 74–78% yield, these processes are still exceeding the common transformations. Shortening the electrolysis time can readily be done by changing to a sandwich like setup. We chose to use two wire cathodes together with the anode plate in between them, thus, doubling the anodic surface area. This led to an improved yield of 84% on 1 g scale (100 mL vessel). Especially, regarding the aforementioned economic benefits and to the scale the presented procedure can be considered as a highly competitive alternative to classical synthesis routes. Following the described

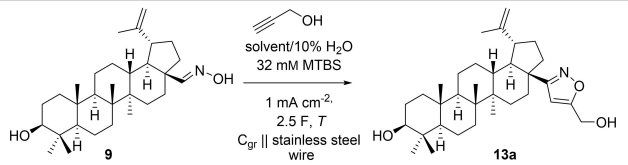
protocol, the 20,29-dihydrogenated nitrile oxide **12** was also synthesized starting from corresponding aldoxime **10**. On 50 mg scale an excellent yield of 96% was reached for the nitrile oxide **12** synthesis (Scheme 6).

The optimized electrochemical oxidation of oxime **9** set the stage for development of a one pot process, which combines synthesis nitrile oxide **11** and the following 1,3-DCR with propargyl alcohol as model alkyne (Table 1). It was found, that 1,3-DCR takes additional 12 h after the completion of electrolysis. An excess of the alkyne (5 equiv.) gave the best results. By changing from methanol to 1-butanol and, thus, enabling heating up to 100 °C, the yield could be further improved (Table 1, entry 6).



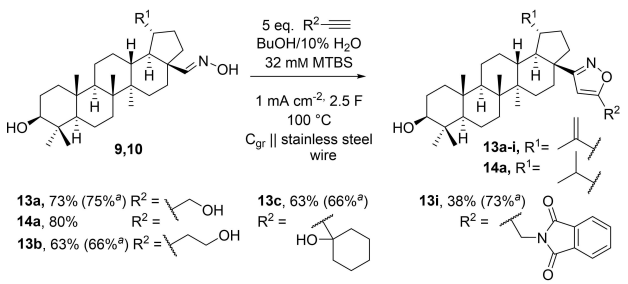
Scheme 6. Electrochemical generation of nitrile oxides **11** and **12** on 50 mg scale. [a] 1000 mg scale.

Table 1. Optimization of the one-pot electrooxidation – 1,3-DCR process towards lupane-type triterpenoid-isoxazole conjugate **13a**.



Entry	Solvent	T [°C]	Propargyl alcohol excess (equiv.)	Additional stirring [h]	Yield, [%]
1	MeOH	50	5	9	54
2	MeOH	50	5	12	66
3	MeOH	50	2	12	61
4	MeOH	50	3	12	59
5	MeOH	50	4	12	63
6	1-BuOH	100	5	12	73
7	^{t}AmOH	100	5	12	41
8	Py	100	5	12	21
9[a]	1-BuOH	50	5	12	75

[a] Alkyne added after electrolysis then heated to 100 °C.



Scheme 7. One-pot electrooxidation – 1,3-DCR process of lupane-type triterpenoid-isoxazole conjugates **13** and **14**. [a] electrolysis at 50 °C, alkyne added after electrolysis, then heated to 100 °C (ex-cell).

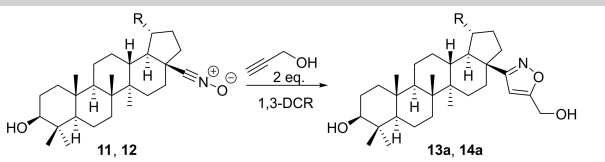
With these parameters conjugates with different alkynes have been synthesized in yields up to 75%, implicating a remarkable quantitative yield for the oxime oxidation step if the same yield as in the classical procedure is assumed for the 1,3-dipolar cycloaddition (Scheme 7). Also an one pot process for oxime **10** electrochemical oxidation and 1,3-DCR reaction with propargyl alcohol was explored and compound **14a** was obtained with 80% yield. For the *N*-propargyl phthalimide derived conjugate the electrochemical in-cell protocol was less efficient. The ex-cell process, however, gave comparable yields to the other alkynes.

To increase the library of products and to improve the yields of the 1,3-DCR, it was decided to separate 1,3-DCR and electrochemical oxidation and to study isoxazole formation as single process (Table 2). 1,3-DCR of propargyl alcohol and nitrile oxides **11** and **12** were performed in CH_2Cl_2 in the presence of Et_3N as a base additive (Table 2). We observed that a successful 1,3-DCR of **12** and alkyne occurs at room temperature in 12 h and the isoxazole **14a** forms in 90% yield (Table 2, Entry 5). Similar reaction without base addition is still successful however gives a lower yield (Table 2, Entry 4).

Surprisingly, identical conditions were suboptimal in the case of the nitrile oxide **11**. The conversion of the latter was incomplete and the expected product **13a** was formed with only 24% yield (Table 2, Entry 1). After increase of both the reaction time and the temperature, a sufficient yield (88%) of **13a** was achieved. A similar yield of **13a** was obtained in 6 h when the reaction proceeded in refluxing pyridine (Table 2, Entry 3). Hence, we have used this procedure for further 1,3-DCR involving nitrile oxide **11**. On the other hand, synthesis of **14a** using 1,3-DCR in refluxing pyridine gave 68% yield. The ^{15}N-labeled isoxazole **13a'** was also synthesized from the nitrile oxide **11'** and a propargyl alcohol *via* 1,3-DCR. The ^{15}N NMR spectrum of **13a'** revealed a −6.53 ppm shift compared to CH_3NO_2 (Figure 4c).

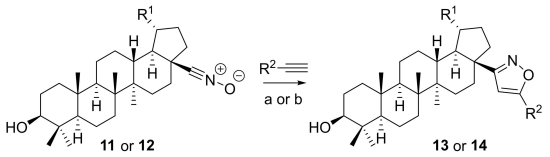
Having optimized the 1,3-DCR conditions between propargyl alcohol and both nitrile oxides **11**, **12**, we expanded the library of lupane-type triterpenoid-isoxazole hybrids. Alkyne components were chosen with such functional groups, which would improve the solubility of the prospective hybrids in aqueous media for their cytotoxicity studies. The cycloaddition reaction is compatible with the selected alkynes and provides

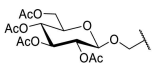
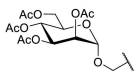
Table 2. Synthesis of isoxazoles **13a**, **14a** from nitrile oxides **11**,**12**.



Entry	Substr.	R	Conditions Solvent	Base	Time [h]	*T* [°C]	Yield, [%]
1			CH_2Cl_2	Et_3N	12	RT	**13a**, 24
2	**11**				24	40	**13a**, 88
3			Py		6	115	**13a**, 89
4			CH_2Cl_2	–	12	RT	**14a**, 81
5	**12**			Et_3N			**14a**, 90
6			Py		6	115	**14a**, 68

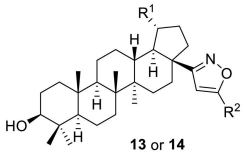
Table 3. Synthesis of lupane-type triterpenoid-isoxazole hybrids from betulin-derived nitrile oxides and alkynes.[a,b]

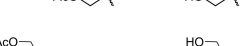
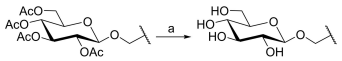
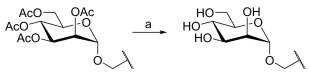
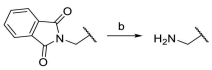


Entry	Substr.	R^1	R^2	Method	Yield of 13 and 14
1	11			a	**13b**, 82%
	12			b	**14b**, 58%
2	11			a	**13c**, 84%
	12			b	**14c**, 69%
3	11			a	**13d**, 79%
	12			b	**14d**, 74%
4	11			a	**13e**, 76%
	12			b	**14e**, 67%
5	11			a	**13f**, 80%
	12			b	**14f**, 72%
6	11			a	**13g**, 72%
	12			b	**14g**, 56%
7	12			b	**14h**, 48%
8	11			a	**13i**, 81%
	12			b	**14i**, 61%

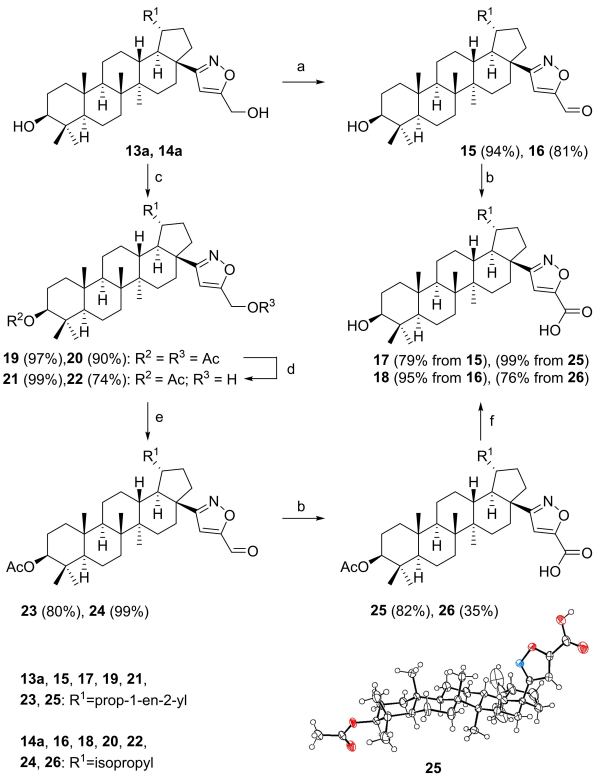
[a] 2 eq. alkyne, Py, 115 °C, 6 h; [b] 2 eq. alkyne, Et_3N, CH_2Cl_2, RT, 12–36 h.

Table 4. Deprotection of lupane-type triterpenoid-isoxazole hybrids.[a,b]



Substr.	R^1	R^2	Yield
14f			**14f'**, 77%
13g			**13g'**, 98%
14g			**14g'**, 73%
14h			**14h'**, 77%
13i			**13i'**, 96%
14i			**14i'**, 60%

[a] NaOMe, MeOH, RT, 2 h. [b] $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 3 h.



Scheme 8. Synthesis of lupane-isoxazole-5-carboxylic acids **17** and **18**. Reagents and conditions: (a) MnO_2, $CHCl_3$, reflux, (6 h for **13a**, 20 h for **14a**), (b) $NaClO_2$, NaH_2PO_4, 2,3-dimethyl-2-butene, THF/**t**-BuOH (1 : 1), RT, 3 h; (c) Ac_2O, DMAP, Py, RT, 18 h; (d) NaOMe, MeOH, RT, 1.5 h; (e) PCC, CH_2Cl_2, RT, 2 h; (f) KOH, EtOH, 50 °C, 15 h. DMAP = 4-dimethylaminopyridine.

the expected isoxazoles in good yields (Table 3). In general, the 1,3-DCR of **12** with the same alkynes required longer reaction times for a full conversion of the starting material than in the case of nitrile oxide **11**.

Next, deprotection of the aforementioned hybrids was performed to obtain triterpene derivatives containing hydrophilic functional groups (Table 4). For this purpose, acetate protected compounds **14f**, **13**–**14g** and **14h** were treated with a catalytic amount of NaOMe in methanol while phthalimides **13i** and **14i** were treated with hydrazine in refluxing ethanol to give amines **13i'** and **14i'**.

In the context of biological activity of triterpenoids, their carboxylic acid derivatives often exhibit higher anticancer and/or antiviral activity. Therefore, our next target was triterpenoids modified with isoxazole-5-carboxylic acid moiety (compounds **17**,**18**, Scheme 8). Synthetic strategy with a selective oxidation of pseudo benzylic alcohol and following Pinnick oxidation seemed the simplest and the fastest route that circumvents the use of toxic chromium reagents in the final stage of the synthesis. First, compounds **13a** and **14a** were treated with MnO_2 in $CHCl_3$ at 60 °C to afford corresponding target aldehydes **15** and **16**. While the aldehyde **15** formed successfully in 6 h, we observed only 55% conversion of alcohol **14a** to **16** under similar conditions. It was necessary to prolong the reaction to 20 h in order to fully consume substrate **14a** and obtain **16** in 81% yield. Unexpectedly, here we also observed oxidation of C(3)-OH and a formation of the ketone as a

byproduct. Furthermore, the synthesis of acids under Pinnick oxidation reaction conditions resulted in inseparable triphasic mixtures. Acids **17** and **18** were obtained in 79% and 95% yields after purification of a dry crude residue of the reaction with a reverse phase column chromatography.

To avoid a solubility problem of the target triterpenoid acids in the Pinnick reaction and obtain additional compounds for cytotoxicity studies, an alternative synthetic route was employed. The synthesis started with acetylation of both hydroxyl groups in compounds **13a** and **14a** under classical Ac_2O/Py/DMAP conditions in order to increase lipophilicity of the molecules. Subsequent selective deacetylation of primary alcohols using NaOMe leads to formation of monoacetylated products **21** and **22**. Next, these compounds were subjected to two-stage oxidation (PCC and Pinnick conditions) and finally the reaction sequence ended by straightforward deprotection of the secondary alcohol that resulted in acids **17** and **18**. Molecular structure of compound **25** was also proved by X-ray analysis.[67]

With the obtained compound library in hand, their *in vitro* cytostatic activity was tested against breast cancer MCF7, glioblastoma multiform cells U-87 MG, lung carcinoma A549, heptocarcinoma HepG2 cell lines using immortalized human fibroblasts hTERT as the non-cancer control. Doxorubicin as a well-known positive control was used. Table 5 represents the cytostatic activity tests of the novel lupane-type triterpenoid-isoxazole hybrids expressed as half-maximal inhibition ($GI_{50} \pm$ SEM, μM).

With few exceptions (**14c**, **13d**, **13f**), it can be emphasized that practically all of the obtained compounds are harmless for human fibroblasts ($GI_{50} > 100$ μM). Among the tested compounds, there is no unambiguous correlation between the compound series bearing isopropenyl substituent (C(20)-C(29)-double bond) at the terpenoid core (**13a–i'**, **17**, **25**) and those possessing isopropyl substituent (**14a–i'**, **18**, **26**). However, we can identify several compounds that exhibit notable cytostatic effect with the GI_{50}.value down to 11 μM and selectivity index 9 (the nonmalignant control (hTERT) $GI_{50} > 100$ μM). Thus, compound **13a** is slightly more toxic towards to lung cancer cell lines A549 (11.05 ± 0.88 μM) while the analogue **14a** showed similar toxicity (14.55 ± 0.92 μM) against U-87 MG cells. Incorporation of CH_2-group between isoxazolyl ring and hydroxyl function (compounds **13b**,**14b**) leads to loss of activity. Compound **14d** and **13e** were toxic to breast cancer MCF7 cells (11.47 ± 0.84 μM) and (14.51 ± 1.42 μM), respectively. On some occasions the presence of acetate protecting group was important for the enhanced activity of the tested compounds. For instance, cytostatic activity of compound **14f** (-CH_2SAc side chain) is around 3 times higher against breast cancer MCF7 (12.49 ± 1.18 μM), glioblastoma U-87 MG (18.16 ± 0.88 μM) and lung cancer A549 (13.15 ± 1.56 μM) cells lines than that of compound **14f'** (–CH_2SH substituent). Also compound **25** (−COOH side chain) containing 3-*O*-acetate exerted higher activity (e.g. GI_{50} (A549) = 11.51 ± 0.88 μM) than its 3-*O*-deacetylated congener **17** (GI_{50} (A549) = 56.24 ± 2.64 μM).

Also artificial saponins containing glucose substituent (**13g'**, **14g'**) revealed cytostatic effect against A549 (15.72 μM) and MCF7 (17.32 μM) cells, respectively. We can conclude that the obtained library of lupane-type triterpenoid-isoxazole conjugates has shown significant cytostatic activity with acceptable selectivity index against MCF7, U-87 MG and A549 cell lines, but were practically inactive against heptocarcinoma HepG2 cell lines. The best cytostatic properties (GI_{50} ~11 μM) were demonstrated by compounds bearing small hydrophilic substituents at the isoxazole ring: hydroxymethyl- (**13a**,**14a**), 2-hydroxypropan-2-yl- (**14d**), (2-hydroxyethoxy)methyl (**13e**), (acetylthio)methyl-groups (**14f**) and carboxylic acid moiety (**25**).

Table 5. Concentrations of half-maximal inhibition ($GI_{50} \pm$SEM, μM) of tested compounds on immortalized human fibroblasts, MCF7, U-87 MG, A549, HepG2 cells.

Compound	Immortalized human fibroblasts	MCF7	U-87 MG	A549	HepG2
doxorubicin	3.15 ± 1.20	4.02 ± 1.07	2.88 ± 0.94	5.02 ± 1.11	10.08 ± 1.84
13a	>100	44.11 ± 2.48	>100	11.05 ± 0.88	>100
14a	82.71 ± 6.01	22.58 ± 3.18	14.55 ± 0.92	44.13 ± 1.88	>100
13b	>100	29.03 ± 1.18	33.45 ± 5.02	30.49 ± 5.07	44.04 ± 2.44
14b	>100	45.22 ± 5.08	23.54 ± 3.04	32.13 ± 0.92	77.18 ± 6.17
13c	>100	42.18 ± .6.77	28.18 ± 3.02	35.44 ± 0.12	40.18 ± 3.81
14c	30.28 ± 4.77	34.55 ± 1.22	48.22 ± 5.66	27.48 ± 2.81	>100
13d	43.15 ± 5.06	24.63 ± 3.20	>100	24.34 ± 3.05	>100
14d	>100	11.47 ± 0.84	21.15 ± 0.71	29.31 ± 0.75	49.65 ± 4.18
13e	>100	14.51 ± 1.42	28.18 ± 1.65	16.61 ± 0.89	78.42 ± 2.81
14e	88.47 ± 10.08	21.15 ± 1.14	32.88 ± 2.12	>100	>100
13f	30.33 ± 5.74	>100	35.18 ± 2.11	30.14 ± 5.72	47.11 ± 1.65
14f	>100	12.49 ± 1.18	18.16 ± 0.88	13.15 ± 1.56	83.41 ± 2.64
14f'	>100	38.51 ± 2.74	>100	87.61 ± 5.72	>100
13g'	>100	92.14 ± 4.18	>100	17.32 ± 0.96	>100
14g'	>100	15.72 ± 0.69	22.14 ± 1.26	>100	65.42 ± 0.86
14h'	>100	42.15 ± 5.89	49.26 ± 1.16	87.17 ± 2.81	>100
13i'	>100	48.23 ± 2.79	>100	>100	64.51 ± 3.92
14i'	>100	31.05 ± 2.84	71.21 ± 4.33	22.89 ± 3.07	>100
17	>100	34.26 ± 1.28	>100	56.24 ± 2.64	>100
18	92.55 ± 5.71	88.45 ± 4.45	52.11 ± 1.22	>100	61.89 ± 2.78
25	>100	18.15 ± 2.11	20.18 ± 1.08	11.51 ± 0.88	81.15 ± 2.98
26	>100	64.12 ± 3.28	>100	24.81 ± 1.06	>100

Conclusion

In summary, we have designed novel semisynthetic lupane-type triterpenoid derivatives containing C–C-bounded isoxazole moiety at the C(17). The developed approach places isoxazole substituent as a linker between triterpenoid scaffold and various substituents of choice. Thus, the isoxazole-linked glucose and mannose conjugates can be regarded as novel artificial saponins.

The key intermediates in the synthetic sequence were isolable nitrile oxides, the stability of which can be explained by the adjacent sterically congested quaternary center. Their synthesis was achieved by TEMPO-mediated electrooxidation of betulin to betulinal, which was optimized for the use of boron-doped diamond electrodes and potassium acetate as supporting electrolyte. Further, a selective direct electrooxidation of betulinal-derived oxime was developed with graphite anode and stainless steel wire as cathode in the presence of methyltributylammonium methylsulfate in the role of supporting electrolyte. It was shown that one-pot aldoxime electrooxidation – 1,3-dipolar cycloaddition is possible and the propargyl alcohol adduct was isolated in up to 80% yield. The developed successful electrooxidation protocols for betulin and betulinal oxime demonstrate that it is possible to overcome the obvious issues of incompatible solubility, which are common for mixing highly lipophilic substrates with polar and ionic electrolysis medium.

On the other hand, stable and isolable nitrile oxides are also suitable for convergent approach, according to which they are employed in 1,3-dipolar cycloaddition as purified distinct reagents. The structures of nitrile oxides have been unambiguously proven by ^{15}N NMR spectra and by single crystal X-ray crystallography. The ^{15}N-labeled compound group "aldoxime – nitrile oxide – isoxazole" contributes original data to the physical organic chemists dealing with nuclear magnetic resonance spectroscopy. The rich chemistry of nitrile oxides (e.g. cycloadditions with alkenes and other unsaturated systems or reactions with nucleophiles) opens a myriad of modification possibilities in the future.

The obtained semisynthetic lupane-type triterpenoid-isoxazole conjugates revealed notable cytostatic activity against breast cancer cell line MCF7, glioblastoma multiform cell line U-87 MG, lung carcinoma cell line A549 and were identified as harmless to immortalized human fibroblasts hTERT as the nonmalignant control. Compounds bearing small hydrophilic substituents at the isoxazole ring possessed he best cytostatic properties (GI_{50} ~11 μM). Finally, we can conclude that here described research demonstrates a successful combination of electroorganic and conventional synthesis of triterpenoid derivatives in the context of medicinal chemistry application.

Experimental Section

Solvents for the reactions were dried over standard drying agents and freshly distilled prior to use. All purchased chemicals (Fluka, Aldrich) were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254} and visualized by using UV lamp. Column chromatography was performed on silica gel (60 Å, 40–63 μm, ROCC). Flash column chromatography was performed on a Büchi Sepacore system (Büchi-Labortechnik GmbH, Essen, Germany) with a Büchi Control Unit C-620, an UV detector Büchi UV photometer C-635, Büchi fraction collector C-660 and two Pump Modules C-605. Fourier transform infrared (FT-IR, Varian 800 FT-IR, Scimitar Series, USA) spectra were recorded in the Attenuated Total Reflectance (ATR, GladiATRTM, Pike technologies, USA) mode. Spectra were obtained at 4 cm^{-1} resolution co-adding 50 scans over a range of wavenumbers from 400 cm^{-1} to 4000 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker 300, 400 and 500 MHz, in $CDCl_3$, [D_6]DMSO, [D_8]THF or [D_4]MeOD at 25 °C. Chemical shifts (δ) values are reported in ppm. The residual solvent peaks are used as internal reference ($CDCl_3$ 7.26 ppm, [D_6]DMSO 2.50 ppm, [D_8]THF 3.58 ppm, [D_4]MeOD 3.31 ppm for ^1H NMR, $CDCl_3$ 77.16 ppm, [D_6]DMSO 39.52 ppm, [D_8]THF 67.57 ppm, [D_4]MeOD 49.00 ppm for ^{13}C NMR), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet); *J* in hertz. High-resolution massspectra (ESI) were performed on Agilent 1290 Infinity series UPLC connected to Agilent 6230 TOF mass spectrometer (calibration at m/z 121.050873 and m/z 922.009798).

The human cancer cells of the glioblastoma (U-87MG), lung cancer cell line (A549), heptocarcinoma (HepG2) cell lines and human breast cancer cells (MCF7) were used in this study. The cells were cultured in the RPMI-1640 medium that contained 10% embryonic calf serum, L-glutamine (2 mmol/L), gentamicin (80 mg/mL) and lincomycin (30 mg/mL) in a CO_2 incubator at 37 °C. The tested compounds were dissolved in DMSO and added to the cellular culture at the required concentrations. Three wells were used for each concentration. The cells which were incubated without the compounds were used as a control. Cells were placed on 96-well microliter plates and cultivated at 37 °C in 5% CO_2/95% air for 72 h. The cell viability was assessed through an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] conversion assay. A total of 1% MTT was added to each well. Four hours later, DMSO was added and mixed for 15 min. Optical density (D) of the samples was measured on a BioRad 680 multi-well spectrophotometer (USA) at the wavelength of 450 nm. The 50% cytotoxic dose GI_{50} of each compound (i.e., the compound concentration that causes the death of 50% of cells in a culture, or decreases the optical density twice as compared to the control wells) was calculated from the data obtained. Statistical processing of the results was performed using the Microsoft Excel-2007, STATISTICA 6.0 and GraphPad Prism 5.0 programs. The results are given as an average value ± a deviation from the average (mean ± standard error of the mean (SEM)). Reliability of differences (p) was estimated using the Student t test. The differences with $p < 0.05$ were considered as reliable. The experimental results are given as the data average values obtained from three independently conducted experiments.[68]

3β-Hydroxylup-20(29)-en-28-al 2:[34] In a 100 mL beaker-type electrolysis cell equipped with heating jacket and reflux condenser a solution of betulin **1** (5.00 g, 11.3 mmol) and KOAc (1.47 g, 15 mmol) in *N,N*-dimethylacetamide (90 mL) and water (10 mL) was heated to 65 °C and TEMPO (265 mg, 1.70 mmol, 15 mol%) was added. The mixture was subjected to electrolysis with a current density of 2.5 mA cm^{-2} at a BDD anode and a copper cathode until 3.33 F (3634 C) were applied. Subsequently TEMPO (265 mg, 1.70 mmol, 15 mol%) was added again and the electrolysis protocol was repeated. The reaction mixture was taken up in water (900 mL) and filtrated with suction. The residue washed with water, air dried and purified by flash column chromatography on silica with *c*-Hex/EtOAc (0%→8% EtOAc) to yield 3.97 g (80%) betulinal **2** as a colorless powder. $R_f = 0.42$ (*c*-Hex/EtOAc 3:1). ^1H and ^{13}C NMR spectra matching those reported in literature.

3β,28-Diacetyloxylup-20(29)-ene 3:[69] Betulin **1** (20 g, 0.045 mol, 1 equiv.) was added to a solution of Ac_2O (18.4 g, 0.18 mol, 4 equiv.) in C2 petrol (200 mL) and heated to reflux for 3 h. The reaction mixture was allowed to cool to rt, and CH_2Cl_2 (40 mL) was added. The residue was washed with water, dried over Na_2SO_4, filtered and evaporated until crystallization began. Product was allowed to crystallize completely, filtered and dried. Yield 22.52 g, 95%. Colorless amorphous product. ^1H and ^{13}C NMR spectra matching those reported in literature.

3β,28-Diacetoxylupane 4:[55] A solution of **3** (5 g, 9.49 mmol, 1 equiv.) in THF (12 mL) was hydrogenated under H_2 (30 bar) over 5% Pd/C (0.2 g, 40 wt%) with stirring for 72 h and then filtered through the celite. Removal of the solvent in vacuo afforded **4** (4.816 g, 96%) as a colorless amorphous product. ^1H and ^{13}C NMR spectra matching those reported in literature.

3β-Acetoxy-lupan-28-ol 5:[55] Compound **4** (10 g, 0.019 mol, 1 equiv.) and $Al(i\text{-}OPr)_3$ (6.482 g, 0.023 mol, 1.4 equiv.) were stirred under reflux in *i*-PrOH (150 mL) for 2 h. The crude mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (100 mL), washed with 5% H_2SO_4 (3×25 mL) and brine (3×20 mL), dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography (20% Hex/EtOAc) giving 8.167 g (89%) of **5** as a colorless amorphous compound. $R_f = 0.58$ (EtOAc/Hex 1:3). ^1H and ^{13}C NMR spectra matching those reported in literature.

Lupan-3β,28-diol 6 starting from compound 1:[41] A solution of **1** (2.00 g, 4.52 mmol, 1 equiv.) in THF (50 mL) and methanol (100 mL) was hydrogenated under H_2 (1 bar) over 10% Pd/C (0.1 g, 5 wt%) with shaking for 144 h and then filtered through the celite. The solvent was removed under reduced pressure. Purification of the crude via flash column chromatography on silica with CH_2Cl_2/EtOAc (0%→8% EtOAc) yielded 1.9 g (95%) of a colorless solid. ^1H and ^{13}C NMR spectra matching those reported in literature.

Lupan-3β,28-diol 6 starting from compound 4:[55] Compound **4** (100 mg, 0.189 mmol, 1 equiv.) was dissolved in EtOH (15 ml) and 1 M KOH (1.1 ml, 5.8 equiv. KOH) aqueous solution was added, reaction was stirred under refluxed for 4 h. Ethanol was removed in vacuo, the residue was suspended in CH_2Cl_2, washed with water, dried over Na_2SO_4, filtered and evaporated. Yield 84 mg, quant. Colorless amorphous product. $R_f = 0.21$ (CH_2Cl_2). ^1H and ^{13}C NMR spectra matching those reported in literature.

3β-Acetyloxy-28-lupanal 7:[70] In a 25 mL beaker-type electrolysis cell equipped with heating jacket and reflux condenser compound **6** (250 mg, 0.562 mmol, 1 equiv.) and tetrabutylammonium acetate (750 mg, 2.5 mmol) were dissolved in 22.5 mL 2,6-lutidine and 2.5 mL water. The mixture was heated to 65 °C and TEMPO (40 mg, 0.256 mmol, 45%) was added. The mixture was subjected to electrolysis with a current density of 2.5 mA cm^{-2} at a BDD anode and a copper cathode until 10 F (542 C) were applied. The mixture was poured into 250 mL 1 M aqueous hydrochloric acid, filtered with suction, washed with water and air dried. Flash column chromatography on silica with *c*-Hex/EtOAc (0%→8% EtOAc) yielded 190 mg (76%) of product **7** as a colorless powder. ^1H and ^{13}C NMR spectra matching those reported in literature.

3β-Hydroxy-28-lupanal 8:[55] In a 5 mL PTFE cell compound **5** (55 mg, 0.113 mmol, 1 equiv.), KOAc (73 mg, 0.75 mmol) and MTBS (25 mg, 0.08 mmol) were dissolved in 5 mL of a 9:1 (v/v) mixture of 2,6-lutidine and water. The mixture was heated to 65 °C and TEMPO (8 mg, 0.051 mmol, 45%) was added. The mixture was subjected to electrolysis with 2.5 mA cm^{-2} at a BDD anode and a copper cathode until 10 F (109 C) were applied. The mixture was poured into 50 mL 1 M aqueous hydrochloric acid, filtered with suction, washed with water and air dried. The crude was purified by flash column chromatography over silica with *c*-Hex/EtOAc (0%→8% EtOAc) to yield 51 mg (93%) of compound **8** as a colorless powder. ^1H and ^{13}C NMR spectra matching those reported in literature.

General procedure for the oxime synthesis

To a solution of KOH (1.91 g, 34.03 mmol, 10 equiv.) in MeOH (10 mL) solution of $NH_2OH \cdot HCl$ (0.94 g, 69.49 mmol, 4 equiv.) in MeOH (10 mL) was added. After formation of colorless precipitate, compound **2** (1.50 g, 3.40 mmol, 1 equiv.) was added to the reaction mixture and the reaction was stirred for 48 h. Reaction mixture was diluted with CH_2Cl_2 (80 mL) and NaHCO3 aq. solution (100 mL), organic phase was washed with brine (3×25 mL), dried over Na_2SO_4, filtered, and evaporated in vacuum. The solid was dried in vacuo to yield oxime **9**[41] as a colorless amorphous product (1.50 g, 97%). $R_f = 0.53$ (CH_2Cl_2/MeOH/24%$NH_3(H_2O)$ 20:1:0.3). ^1H and ^{13}C NMR spectra matching those reported in literature.

(*E*)-3β-Hydroxylup-20(29)-en-28-aldoxime (^{15}N) 9′: According to general procedure compound **9′** was prepared from compound **2** (100 mg, 0.227 mmol, 1 equiv.), $^{15}NH_2OH \cdot HCl$ (32 mg, 0.454 mmol, 2 equiv.), KOH (64 mg, 1.135 mmol, 5 equiv.). Yield 102 mg, 98%. Colorless amorphous product. $R_f = 0.35$ (CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.55$ (d, $^2J_{HN} = 2.2$ Hz, 1H), 4.71 (s, 1H), 4.60 (s, 1H), 3.19 (dd, $^3J = 11.4$, 4.8 Hz, 1H), 2.51 (td, $^3J = 11.1$, 5.7 Hz, 1H), 2.01–1.89 (m, 2H), 1.86–1.14 (m, 22H, including s: 1.69 CH_3), 1.13–0.72 (m, 18H, including 5 s: 0.99, 0.98, 0.96, 0.82, 0.76, CH_3), 0.67 (d, $^3J = 9.3$ Hz; 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 155.89$ (d, $^1J_{NC} = 2.2$ Hz), 149.96, 110.20, 79.16, 55.44, 50.53, 49.92 (d, $^2J_{NC} = 6.4$ Hz), 49.50, 48.02, 42.99, 41.03, 39.00, 38.84, 38.73, 37.31, 37.16, 34.44, 32.53, 29.87, 28.14, 28.02, 27.52, 25.35, 20.93, 19.32, 18.43, 16.24, 16.19, 15.52, 14.89; ^{15}N NMR (51 MHz, $CDCl_3$): $\delta = -25.61$; IR (neat): 3310, 2935, 2865, 1450, 1375, 1260, 1105, 1040, 1030, 1010, 905, 885, 730 cm^{-1}.

3β-Hydroxylupan-28-aldoxime 10: According to general procedure compound **10** was prepared from compound **7** (4.00 g, 8.25 mmol, 1 equiv.), $NH_2OH \cdot HCl$ (2.096 g, 33.01 mmol, 4 equiv.), KOH (4.629 g, 82.52 mmol, 10 equiv.). Yield (4.09 g, 99%). Colorless amorphous product. $R_f = 0.39$ (EtOAc/Hex 1:9). ^1H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.47 (s, 1H), 3.19 (dd, $^3J = 10.9$, 5.1 Hz, 1H), 2.05–1.99 (m, 1H), 1.94–1.10 (m, 22H), 1.09–0.61 (m, 24H including 4 s: 0.97 (6H), 0.95, 0.77, 0.76 CH_3 and 0.86 (d, $^3J = 6.7$ Hz, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 156.43$, 79.13, 55.36, 50.43, 50.18, 48.77, 44.79, 43.20, 41.00, 38.99, 38.79 (2C), 38.06, 37.26, 34.49, 32.07, 29.62, 28.13, 27.87, 27.50, 26.89, 23.06, 22.08, 20.89, 18.43, 16.17 (2C), 15.53, 15.02, 14.80; IR (neat): 3400, 2950, 2925, 2860, 1465, 1445, 1385, 1365, 1275, 1180, 1075, 1025, 1005, 930, 870 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{30}H_{51}NO_2 + H]^+$ 458.3993; found 458.3968.

(17S)-17-{[3β-Hydroxy-28-norlup-20(29)-en]}-nitrile oxide 11: Conventional oxidative dehydration: To the solution of betulin oxime **9** (0.630 g, 1.382 mmol, 1 equiv.) in acetone (20 mL) PIFA (0.713 g, 1.659 mmol, 1.2 equiv.) was added. Once the reaction was complete, TLC (CH_2Cl_2/EtOAc 4:1), saturated water solution of $NaHCO_3$ (2 mL) was added and reaction mixture was evaporated. Ethyl acetate (50 mL) was added to the reaction; the mixture was washed with saturated aq. solution $NaHCO_3$ (2×6 mL) and brine (5×6 mL), dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→20% EtOAc) giving 439 mg (70%) of nitrile oxide **11**.

Electrochemical oxidative dehydration: In a 100 mL beaker-type electrolysis cell equipped with heating jacket and reflux condenser betulin oxime **9** (1.00 g, 2.19 mmol, 1 equiv.) and MTBS (1.00 g, 3.21 mmol) were dissolved in 90 mL methanol and 10 mL water. The mixture was heated to 50 °C and subjected to electrolysis with a current density of 1 mA cm^{-2} at an isostatic graphite anode in

between two stainless steel wire spirals as cathodes until 2.5 F (528 C) were applied. The solvent was evaporated under reduced pressure. Flash column chromatography over silica with CH_2Cl_2/EtOAc (0%→2% EtOAc) yielded 838 mg (84%) nitrile oxide **11**.

R_f = 0.73 (CH_2Cl_2/EtOAc 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.75 (s, 1H), 4.65 (s, 1H), 3.18 (dd, ^3J = 11.4, 4.8 Hz; 1H), 2.56 (td, ^3J = 10.9, 5.8 Hz; 1H), 2.16–2.06 (m, 1H), 2.06–1.96 (m, 2H), 1.83–1.18 (m, 21H; including s, 1.67 CH_3), 1.15–0.73 (m, 17H; including 5 s: 1.07, 0.97, 0.94, 0.84, 0.77, CH_3), 0.68 (d, ^3J = 9.6 Hz; 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 148.25, 111.10, 79.09, 55.47, 51.84, 50.57, 50.27, 49.08, 42.43, 41.94, 40.80, 39.01, 38.88, 37.33, 36.92, 34.50, 32.29, 29.80, 29.60, 28.13, 27.53, 25.10, 20.82, 19.56, 18.40, 16.30, 16.19, 15.50, 15.05; (missing B–C≡N^+–O^-); IR (neat): 3495, 2940, 2870, 2275, 1450, 1375, 1240, 1195, 1030, 885, 755 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{47}NO_2 + NH_4]^+$ 471.3945; found 471.3940.

(17S)-17-{[3β-Hydroxy-28-norlup-20(29)-en]}-(^{15}N)-nitrile oxide 11′: Obtained by similar conventional oxidative dehydration procedure as compound **11**: Compound **9′** (90 mg, 0.197 mmol, 1 equiv.), PIFA (105 mg, 0.244 mmol, 1.2 equiv.), acetone (3 mL). Yield 51 mg, 57%. Colorless amorphous product. ^{1}H NMR (500 MHz, $CDCl_3$) is identical to compound **11**. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 148.25, 111.10, 79.09, 55.47, 51.83, 50.57, 50.27, 49.08, 42.43, 41.94, 41.49 (d, $^1J_{NC}$ = 69.7 Hz), 40.80, 39.01, 38.89, 37.33, 36.92, 34.50, 32.29, 29.80, 29.60, 28.13, 27.53, 25.10, 20.82, 19.56, 18.40, 16.30, 16.19, 15.50, 15.05; ^{15}N NMR (51 MHz, $CDCl_3$): δ = −185.95; IR (neat): 3445, 2935, 2865, 2235, 1450, 1375, 1260, 1240, 1195, 1100, 1025, 800 cm^{-1}.

(17S)-17-{[3β-Hydroxy-28-norlupane]}-nitrile oxide 12: Conventional oxidative dehydration: To the solution of 20,29-dihydro betulin oxime **10** (300 mg, 0.655 mmol, 1 equiv.) and Py (0.026 mL, 0.032 mmol, 0.05 equiv.) in CH_2Cl_2 (5 mL) NCS (0.087 g, 0.655 mmol, 1 equiv.) was slowly added. Reaction was stirred for 12 h at room temperature, ethyl acetate (50 mL) was added to the reaction; the mixture was washed with 20% $CuSO_4 \cdot 5H_2O$ aq. solution (3×10 mL) and brine (3×10 mL), dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography over silica with Hex/EtOAc (0%→10% EtOAc) giving 236 mg (79%) of nitrile oxide **12** as an amorphous compound.

Electrochemical oxidative dehydration: In a 5 mL PTFE cell, 20,29-dihydro betulin aldoxime **10** (50 mg, 0.109 mmol, 1 equiv.) and MTBS (50 mg, 0.160 mmol) were dissolved in a mixture of 4.5 mL methanol and 0.5 mL water. The mixture was heated to 50 °C and subjected to electrolysis with a current density of 1 mA cm^{-2} at an isostatic graphite anode and a stainless steel wire cathode until 2.5 F (26.4 C) were applied. The solvent was evaporated under reduced pressure. Flash column chromatography on silica with CH_2Cl_2/EtOAc (0%→8% EtOAc) yielded 48 mg (96%) nitrile oxide **12**.

R_f = 0.47 (Hex/EtOAc 9:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.20 (dd, ^3J = 10.5, 3.6 Hz; 1H), 2.01–1.48 (m, 15H), 1.47–1.16 (m, 10H), 1.15–0.61 (m, 23H; including 5 s: 1.08, 0.98, 0.93, 0.86, 0.78, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 79.07, 55.42, 52.39, 50.23, 49.95, 45.63, 42.68, 41.89, 40.78, 39.00, 38.86, 37.38, 37.30, 34.56, 32.28, 29.48, 29.44, 28.13, 27.50, 26.49, 22.83, 22.54, 20.79, 18.40, 16.22, 16.18, 15.51, 15.17, 14.93 (missing B–C≡N^+-O^-); IR (neat): 3500, 2950, 2865, 2275, 1740, 1450, 1385, 1240, 1205, 1045, 985 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{30}H_{40}NO_2 + H]^+$ 456.3836; found 456.3816.

Method I: In-cell electrochemical oxidation and 1,3-DCR sequence

In 5 mL PTFE cells betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and the respective alkyne (0.550 mmol, 5 equiv.) were dissolved in 5 mL of a 9:1 (v/v) mixture of butanol and water. The mixture was heated to 100 °C and subjected to electrolysis with 1 mA cm^{-2} at an isostatic graphite anode and a stainless steel wire cathode until 2.5 F (26.5 C) were applied. Afterwards, the mixture was stirred at 100 °C for 12 h. The solvent was evaporated under reduced pressure and the product mixture was purified by flash column chromatography over silica with CH_2Cl_2/EtOAc (0%→8% EtOAc).

Method II: Ex-cell electrochemical oxidation and 1,3-DCR sequence

In 5 mL PTFE cells betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) were dissolved in 5 mL of a 9:1 (v/v) mixture of butanol and water. The mixture was heated to 50 °C and subjected to electrolysis with 1 mA cm^{-2} at an isostatic graphite anode and a stainless steel wire cathode until 2.5 F (26.5 C) were applied. Afterwards, the respective alkyne (0.550 mmol, 5 equiv.) was added, the mixture was heated up and stirred at 100 °C for 12 h. The solvent was evaporated under reduced pressure and the product mixture was purified by flash column chromatography over silica with CH_2Cl_2/EtOAc (0%→8% EtOAc).

Method III: Conventional preparation of isoxazoles 13 a–i

To a solution of betulin nitrile oxide **11** (35 mg, 0.077 mmol, 1 equiv.) in dry Py (1 mL) alkyne (0.231 mmol, 3 equiv.) was added and the reaction mixture was refluxed for 6 h. After cooling, most of the Py was removed under reduce pressure. The residue was dissolved in EtOAc (25 mL), washed with 2% HCl aq. solution (10× 5 mL), saturated aq. solution $NaHCO_3$ (2×3 mL) and brine (5× 3 mL), dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography on silica.

(17S)-17-(5-Hydroxymethyl-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13 a:1) According to method I compound **13 a** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and prop-2-yn-1-ol (32 μL, 0.550 mmol, 5 equiv.). Yield 41 mg, 73%.

2) According to method II compound **13 a** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and prop-2-yn-1-ol (32 μL, 0.550 mmol, 5 equiv.). Yield 42 mg, 75%.

3) According to method III compound **13 a** was prepared from betulin nitrile oxide **11** (430 mg, 0.948 mmol, 1 equiv.), prop-2-yn-1-ol (168 μL, 2.843 mmol, 3 equiv.), Py (10 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc). Yield 432 mg, 89%. Colorless solid.

R_f = 0.63 (Hex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.11 (s, 1H), 4.77 (s, 3H), 4.61 (dq, ^2J = 2.8 Hz, ^4J = 1.4 Hz, 1H), 3.18 (dd, ^3J = 11.4, 4.8 Hz, 1H), 3.05 (td, ^3J = 11.0, 4.4 Hz; 1H), 2.48 (td, ^3J = 12.5, 3.5 Hz; 1H), 2.05 (dt, ^2J = 13.1 Hz, ^3J = 3.1 Hz; 1H), 1.86–1.17 (m, 21H; including s, 1.72 CH_3), 1.13–0.874 (m, 9H; including 2 s, 1.00, 0.95 CH_3), 0.84–0.72 (m, 9H; including 3 s, 0.80, 0.79, 0.74 CH_3), 0.66 (d, ^3J = 9.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 169.89, 168.11, 150.85, 109.75, 101.41, 79.17, 56.91, 55.50, 50.73, 50.16, 49.93, 46.95, 42.87, 40.95, 39.94, 38.99, 38.85, 37.65, 37.35, 34.66, 34.49, 30.43, 28.72, 28.12, 27.55, 25.56, 21.03, 19.71, 18.43, 16.24, 16.18, 15.49, 15.05; IR (neat): 3315, 2935, 2865, 1590, 1445, 1070, 900, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{51}NO_3 + H]^+$ 510.3942; found 510.3945.

(17S)-17-(5-Hydroxymethyl-isoxazol-3-yl-2-^{15}N)-28-norlup-20(29)-en-3β-ol 13a′: According to method III compound **13′** was prepared

from betulin nitrile oxide **11'** (40 mg, 0.087 mmol, 1 equiv.), prop-2-yn-1-ol (15 μL, 0.262 mmol, 3 equiv.), Py (1 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc). Yield 39 mg, 87%. Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) is identical to compound **13a**. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 169.86, 168.08 (d, $^1J_{NC}$ = 5.5 Hz), 150.85, 109.76, 101.41, 79.17, 56.92, 55.51, 50.73, 50.15 (d, $^2J_{NC}$ = 4.6 Hz), 49.93, 46.95, 42.88, 40.96, 39.95, 39.00, 38.85, 37.65, 37.35, 34.66, 34.49, 30.43, 28.72, 28.13, 27.56, 25.56, 21.03, 19.71, 18.43, 16.24, 16.19, 15.50, 15.05; ^{15}N NMR (51 MHz, $CDCl_3$): δ = −6.53; IR (neat): 3310, 2935, 2865, 1450, 1375, 1260, 1105, 1040, 1030, 1010, 980, 910, 885, 730 cm^{-1}.

(17S)-17-(5-(2-Hydroxyeth-1-yl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13b: 1) According to method I compound **13b** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and but-3-yn-1-ol (42 μL, 0.550 mmol, 5 equiv.). Yield 36 mg, 63%.

2) According to method II compound **13b** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and but-3-yn-1-ol (42 μL, 0.550 mmol, 5 equiv.). Yield 38 mg, 66%.

3) According to method III compound **13b** was prepared from betulin nitrile oxide **11** (86 mg, 0.189 mmol, 1 equiv.), but-3-yn-1-ol (43 μL, 0.568 mmol, 3 equiv.), Py (2 mL). Purified by column chromatography over silica with Hex/EtOAc (0%→10% EtOAc). Yield 81 mg, 82%. Colorless solid.

R_f = 0.46 (Hex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.95 (s, 1H), 4.76 (s, 1H), 4.60 (s, 1H), 3.96 (t, ^3J = 6.2 Hz; 2H), 3.17 (dd, ^3J = 11.4, 4.6 Hz; 1H), 3.05 (td, ^3J = 10.9, 4.3 Hz; 1H), 3.01 (t, ^3J = 6.3 Hz, 2H), 2.46 (td, ^3J = 12.8, 2.7 Hz; 1H), 2.04 (dt, ^2J = 13.2 Hz, ^3J = 3.6 Hz; 1H), 1.86–1.16 (m, 23H; including s: 1.72 CH_3), 1.14–0.84 (m, 9H; including 2 s: 0.99, 0.95 CH_3), 0.84–0.71 (m, 9H; including 2 s: 0.79, 0.74 CH_3), 0.66 (d, ^3J = 9.7 Hz; 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.94, 168.17, 150.93, 109.70, 101.51, 79.16, 60.45, 55.52, 50.74, 50.01 (2C), 47.02, 42.86, 40.96, 39.91, 39.00, 38.85, 37.65, 37.36, 34.68, 34.49, 30.50, 30.44, 28.76, 28.13, 27.57, 25.56, 21.04, 19.72, 18.43, 16.23, 16.14, 15.50, 15.05; IR (neat): 3590, 3345, 2940, 2860, 1605, 1450, 1375, 1045, 1010, 880, 860, 795 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{34}H_{53}NO_3+H]^+$ 524.4098; found 524.4108.

(17S)-17-(5-(1-Hydroxycyclohexyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13c: 1) According to method I compound **13c** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and 1-ethynylcyclohexan-1-ol (71 μL, 0.550 mmol, 5 equiv.). Yield 40 mg, 63%.

2) According to method II compound **13c** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and 1-ethynylcyclohexan-1-ol (71 μL, 0.550 mmol, 5 equiv.). Yield 42 mg, 66%.

3) According to method III compound **13c** was prepared from betulin nitrile oxide **11** (71 mg, 0.156 mmol, 1 equiv.), 1-ethynylcyclohexan-1-ol (60 μL, 0.469 mmol, 3 equiv.), Py (2 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc). Yield 76 mg, 84%. Colorless solid.

R_f = 0.43 (Hex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.02 (s, 1H), 4.76 (s, 1H), 4.60 (s, 1H), 3.17 (dd, ^3J = 11.4, 4.7 Hz; 1H), 3.06 (td, ^3J = 11.1, 4.5 Hz; 1H), 2.43 (td, ^3J = 12.5, 3.1 Hz; 1H), 2.06 (dt, ^2J = 13.3 Hz, ^3J = 3.6 Hz; 1H), 2.03–1.93 (m, 2H), 1.91–1.17 (m, 29H; including s, 1.72 CH_3), 1.12–0.84 (m, 9H; including 2 s: 0.99, 0.95 CH_3), 0.82–0.70 (m, 9H; including 3 s: 0.77, 0.73 CH_3), 0.66 (d, ^3J = 10.1 Hz; 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 176.45, 167.86, 150.92, 109.69, 99.15, 79.16, 70.54, 55.50, 50.71, 50.09, 50.07, 47.08, 42.85, 40.94, 39.82, 39.00, 38.84, 37.67, 37.35, 36.90, 36.85, 34.68, 34.46, 30.51, 28.75, 28.12, 27.56, 25.54, 25.33, 21.91, 21.88, 21.03, 19.75, 18.42, 16.22, 16.02, 15.49, 15.06; IR (neat): 3345, 3290, 2940, 2845, 1580, 1445, 1045, 990, 990, 795 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{38}H_{59}NO_3+H]^+$ 578.4568; found 578.4574.

(17S)-17-(5-(2-Hydroxypropan-2-yl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13d: According to method III compound **13d** was prepared from betulin nitrile oxide **11** (71 mg, 0.156 mmol, 1 equiv.), 2-methylbut-3-yn-2-ol (46 μL, 0.469 mmol, 3 equiv.), Py (2 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc). Yield 66 mg, 79%. Colorless solid. R_f = 0.35 (Hex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.00 (s, 1H), 4.76 (s, 1H), 4.60 (s, 1H), 3.17 (dd, ^3J = 11.4, 4.7 Hz; 1H), 3.05 (td, ^3J = 11.1, 4.5 Hz; 1H), 2.45 (td, ^3J = 12.5, 3.3 Hz; 1H), 2.05 (dt, ^2J = 13.3 Hz, ^3J = 3.5 Hz; 1H), 1.89–1.17 (m, 27H; including 2 s, 1.72, 1.62 CH_3), 1.12–0.84 (m, 9H; including 2 s: 0.99, 0.95 CH_3), 0.83–0.70 (m, 9H; including 2 s: 0.79, 0.73 CH_3), 0.69–0.63 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 176.53, 167.93, 150.89, 109.71, 98.57, 79.15, 69.38, 55.50, 50.72, 50.09, 50.05, 47.03, 42.84, 40.93, 39.83, 38.99, 38.84, 37.64, 37.34, 34.66, 34.46, 30.47, 29.28, 29.24, 28.74, 28.12, 27.55, 25.53, 21.03, 19.72, 18.41, 16.21, 16.04, 15.49, 15.04; IR (neat): 3360, 3310, 2940, 2870, 1580, 1465, 1450, 1380, 1260, 1190, 1045, 970, 895, 795 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{55}NO_3+H]^+$ 538.4255; found 538.4270.

(17S)-17-(5-((2-Hydroxyethoxy)methyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13e: According to method III compound **13e** was prepared from betulin nitrile oxide **11** (61 mg, 0.134 mmol, 1 equiv.), 2-(prop-2-yn-1-yloxy)ethan-1-ol (40 mg, 0.403 mmol, 3 equiv.), Py (2 mL). Purified by column chromatography on silica with Hex/EtOAc (10%→50% EtOAc). Yield 56 mg, 76%. Colorless solid. R_f = 0.39 (Hex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.12 (s, 1H), 4.76 (s, 1H), 4.66 (s, 2H), 4.61 (s, 1H), 3.82–3.75 (m, 2H), 3.70–3.64 (m, 2H), 3.17 (dd, ^3J = 11.4, 4.8 Hz; 1H), 3.06 (td, ^3J = 11.1, 4.5 Hz; 1H), 2.47 (td, ^3J = 12.6, 3.5 Hz; 1H), 2.05 (dt, ^2J = 13.4 Hz, ^3J = 3.2 Hz; 1H), 1.83–1.21 (m, 21H, including s: 1.71 CH_3), 1.11–0.84 (m, 9H, including 2 s: 1.00, 0.95 CH_3), 0.83–0.71 (m, 9H, including 2 s: 0.79, 0.74 CH_3), 0.66 (d, ^3J = 9.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.07, 167.65, 150.85, 109.75, 102.54, 79.15, 72.41, 64.26, 61.93, 55.51, 50.73, 50.12, 49.95, 46.96, 42.87, 40.95, 39.92, 39.00, 38.85, 37.65, 37.35, 34.65, 34.48, 30.44, 28.73, 28.12, 27.56, 25.56, 21.03, 19.72, 18.43, 16.24, 16.12, 15.49, 15.05; IR (neat): 3430, 2935, 2865, 1450, 1360, 1105, 1070, 1045, 1010, 980, 880, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{55}NO_4+Na]^+$ 576.4023; found 576.4035.

(17S)-17-(5-((Acetylthio)methyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13f: According to method III compound **13f** was prepared from betulin nitrile oxide **11** (61 mg, 0.134 mmol, 1 equiv.), *S*-(prop-2-yn-1-yl) ethanethioate (46 mg, 0.403 mmol, 3 equiv.), Py (2 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc). Yield (61 mg, 80%). Colorless solid. R_f = 0.41 (Hex/EtOAc 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.01 (s, 1H), 4.75 (s, 1H), 4.60 (s, 1H), 4.20 (d, ^2J = 15.2 Hz; 1H), 4.16 (d, ^2J = 15.2 Hz; 1H), 3.17 (dd, ^3J = 11.4, 4.8 Hz; 1H), 3.03 (td, ^3J = 11.1, 4.5 Hz; 1H), 2.43 (td, ^3J = 12.5, 3.5 Hz; 1H), 2.39 (s, 3H), 2.01 (2.01 (dt, ^2J = 13.3 Hz, ^3J = 3.1 Hz; 1H), 1.83–1.19 (m, 21H, including s: 1.71 CH_3), 1.13–0.82 (m, 9H, including 2 s: 0.98, 0.95 CH_3), 0.82–0.71 (m, 9H; including 3 s: 0.79, 0.78, 0.74 CH_3), 0.66 (d, ^3J = 9.1 Hz; 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 193.89, 168.36, 166.96, 150.87, 109.70, 102.34, 79.15, 55.51, 50.73, 50.07, 49.98, 46.95, 42.84, 40.94, 39.84, 38.99, 38.85, 37.62, 37.35, 34.60, 34.48, 30.44, 30.40, 28.70, 28.12, 27.56, 25.55, 23.93, 21.03, 19.71, 18.43, 16.23, 16.09, 15.49, 15.04; IR (neat): 3440, 2935, 2865, 1700, 1605, 1450, 1390, 1375, 1355, 1130, 1045, 1005, 880, 800, 755, 620 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{53}NO_3S+H]^+$ 568.3819; found 568.3820.

(17S)-17-(5-(2,3,4,6-Tetra-*O*-acetyl-β-D–gluco-pyranosyloxymethyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13g: According to method III compound **13g** was prepared from betulin nitrile oxide

11 (100 mg, 0.220 mmol, 1 equiv.), propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (170 mg, 0.440 mmol, 2 equiv.), Py (4 mL). Purified by column chromatography on silica with Hex/EtOAc (10%→50% EtOAc). Yield (134 mg, 72%). Colorless solid. R_f = 0.42 (Hex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.10 (s, 1H), 5.21 (dd, ^3J = 9.5, 9.4 Hz; 1H), 5.11 (dd, ^3J = 9.7, 9.5 Hz; 1H), 5.04 (dd, ^3J = 9.4, 8.0 Hz; 1H), 4.87 (d, ^2J = 13.8 Hz; 1H), 4.75 (s, 1H), 4.74 (d, ^2J = 13.8 Hz; 1H), 4.62 (d, ^3J = 8.0 Hz; 1H), 4.61 (s, 1H), 4.28 (dd, ^2J = 12.4 Hz; ^3J = 4.6 Hz; 1H), 4.15 (dd, ^2J = 12.3 Hz; ^3J = 2.1 Hz; 1H), 3.72 (ddd, ^3J = 9.9, 4.5, 2.3 Hz; 1H), 3.17 (dd, ^3J = 11.4, 4.7 Hz; 1H), 3.03 (td, ^3J = 11.0, 4.4 Hz; 1H), 2.45 (td, ^3J = 12.5, 3.5 Hz; 1H), 2.10 (s, 3H, Ac), 2.07–1.98 (m, 10H, including 2 s: 2.02, 2.00 Ac), 1.83–1.21 (m, 21H, including s: 1.72, CH_3), 1.11–0.85 (m, 9H, including 2 s: 1.00, 0.95, CH_3), 0.83–0.71 (m, 9H, including 2 s: 0.80, 0.74, CH_3), 0.66 (d, ^3J = 9.3 Hz; 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.77, 170.32, 169.54, 169.48, 168.12, 166.61, 150.71, 109.81, 103.04, 100.05, 79.12, 72.77, 72.20, 71.19, 68.36, 61.90, 61.89, 55.49, 50.69, 50.13, 49.90, 46.97, 42.86, 40.95, 39.92, 38.99, 38.83, 37.67, 37.34, 34.60, 34.46, 30.40, 28.69, 28.11, 27.54, 25.53, 21.01, 20.88, 20.77, 20.74, 20.72, 19.68, 18.39, 16.21 (2C), 15.48, 15.04; IR (neat): 2940, 2865, 1745, 1365, 1215, 1035, 980, 905, 800, 695 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{47}H_{69}NO_{12}+H]^+$ 840.4893; found 840.4894.

(17S)-17-(5-((1,3-Dioxoisoindolin-2-yl)methyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13i: 1) According to method I compound **13i** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and 2-(prop-2-yn-1-yl) isoindoline-1,3-dione (102 mg, 0.550 mmol, 5 equiv.). Yield 27 mg, 38%.

2) According to method II compound **13i** was prepared from betulin oxime **9** (50 mg, 0.110 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and 2-(prop-2-yn-1-yl)isoindoline-1,3-dione (102 gm, 0.550 mmol, 5 equiv.). Yield 51 mg, 73%.

3) According to method III compound **13i** was prepared from nitrile oxide **11** (100 mg, 0.220 mmol, 1 equiv.), 2-(prop-2-yn-1-yl) isoindoline-1,3-dione (82 mg, 0.440 mmol, 2 equiv.), Py (3.5 mL). Purified by column chromatography on silica with Hex/EtOAc (10%→50% EtOAc). Yield 114 mg, 81%. Colorless amorphous product.

R_f = 0.33 (Hex/EtOAc 3:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.92–7.87 (m, 2H, Ar-H), 7.81–7.69 (m, 2H, Ar-H), 6.09 (s, 1H), 5.02–4.91 (m, 2H), 4.73 (s, 1H), 4.58 (s, 1H), 3.17 (dd, ^3J = 11.4, 4.7 Hz; 1H), 3.01 (td, ^3J = 11.1, 4.6 Hz; 1H), 2.43 (td, ^3J = 12.6, 3.4 Hz; 1H), 1.99 (dt, ^2J = 13.2 Hz, ^3J = 3.2 Hz; 1H), 1.80–1.19 (m, 21H, including s: 1.69 CH_3), 1.08–0.83 (m, 9H, including 2 s: 0.97, 0.94 CH_3), 0.81–0.71 (m, 9H, including 3 s: 0.77, 0.76, 0.73 CH_3), 0.65 (d, ^3J = 9.2 Hz; 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.30 (2C), 167.36, 165.03, 150.85, 134.46 (2C), 132.07 (2C), 123.83 (2C), 109.70, 102.48, 79.16, 55.50, 50.73, 50.09, 49.97, 46.88, 42.82, 40.93, 39.80, 38.99, 38.84, 37.58, 37.33, 34.57, 34.46, 33.36, 30.39, 28.69, 28.12, 27.56, 25.53, 21.01, 19.70, 18.42, 16.23, 16.11, 15.49, 15.01; IR (neat): 2925, 2865, 1775, 1715, 1610, 1420, 1390, 1345, 1100, 1045, 1005, 945, 880, 710 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{41}H_{54}N_2O_4+H]^+$ 639.4156; found 639.4194.

(17S)-17-(5-(β-D-Gluco-pyranosyloxymethyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13g′: To a solution of compound **13g** (115 mg, 0.137 mmol, 1 equiv.) in MeOH (3 mL) NaOMe (2 mg, 0.027 mmol, 0.2 equiv.) was added. Reaction was stirred for 2 h at room temperature, the reaction was quenched by addition of ion-exchange resin (Dowex 50WX8, 50–100 mesh, H− form) till pH 7, filtered and evaporated in vacuum. The residue was purified by column chromatography on silica with CH_2Cl_2/MeOH (0%→3% MeOH). Yield (90 mg, 98%). Colorless solid. R_f = 0.6 (CH_2Cl_2/MeOH 4:1); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 6.51 (s, 1H), 5.15 (d, ^3J = 4.9 Hz; 1H, OH), 4.99 (d, ^3J = 4.6 Hz; 1H, OH), 4.94 (d, ^3J = 5.1 Hz; 1H, OH), 4.87 (d, ^2J = 13.6 Hz; 1H), 4.71 (d, ^2J = 13.6 Hz; 1H), 4.72 (s, 1H), 4.58 (s, 1H), 4.54 (t, ^3J = 5.9 Hz; 1H, OH), 4.25 (d, ^3J = 5.1 Hz; 1H), 4.22 (d, ^3J = 7.8 Hz; 1H), 3.73–3.65 (m, 1H), 3.49–3.41 (m, 1H), 3.17–3.08 (m, 2H), 3.05 (dd, ^3J = 9.0, 4.9 Hz; 1H), 3.03–2.92 (m, 3H), 2.39 (td, ^3J = 12.3, 3.6 Hz; 1H), 2.05 (dt, ^2J = 13.2 Hz; ^3J = 3.4 Hz; 1H), 1.75 (t, ^3J = 11.4 Hz; 1H), 1.72–1.51 (m, 9H, including s: 1.69, CH_3), 1.49–1.07 (m, 11H), 1.05–0.92 (m, 5H, including s: 0.96, CH_3), 0.90–0.78 (m, 4H, including s: 0.86, CH_3), 0.74 (s, CH_3), 0.72 (s, CH_3), 0.67–0.58 (m, 4H, including s: 0.63, CH_3); ^{13}C NMR (126 MHz, [D_4]MeOD): δ = 169.47, 169.20, 151.96, 110.30, 104.33, 103.63, 79.67, 78.20, 78.00, 74.95, 71.59, 62.79, 62.37, 56.86, 51.99, 51.35, 51.00, 48.32, 43.86, 42.06, 40.77, 40.07, 39.94, 38.98, 38.33, 35.57, 35.38, 31.34, 29.75, 28.61, 28.04, 26.74, 22.08, 19.71, 19.42, 16.71 (2C), 16.10, 15.33; IR (neat): 3290, 2935, 2865, 1640, 1445, 1370, 1075, 1030, 1010, 980, 880 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{39}H_{61}NO_8+H]^+$ 672.4470; found 672.4474.

(17S)-17-(5-(Aminomethyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 13i′: $NH_2NH_2 \cdot H_2O$ (22 μL, 0.704 mmol, 5 equiv.) is added to solution of **13i** (0.09 g, 0.141 mmol, 1 equiv.) in EtOH (3 mL) reaction is heated to reflux for 2 h. After cooling, saturated NH_4Cl solution (0.2 mL) is added, reaction mixture is evaporated and purified by column chromatography on silica with CH_2Cl_2/MeOH (0%→3% MeOH). Yield (69 mg, 96%). Colorless amorphous powder. R_f = 0.59 (CH_2Cl_2/MeOH 4:1); ^{1}H NMR (500 MHz, [D_4]MeOD): δ = 6.25 (s, 1H), 4.75 (s, 1H), 4.62 (s, 1H), 3.91 (s, 2H), 3.13 (dd, ^3J = 11.4, 4.8 Hz; 1H), 3.07 (td, ^3J = 10.9, 4.9 Hz; 1H), 2.52 (td, ^3J = 12.6, 3.5 Hz; 1H), 2.12 (dt, ^2J = 13.3 Hz, ^3J = 3.5 Hz; 1H), 1.86 (t, ^3J = 11.5 Hz; 1H), 1.81–1.23 (m, 20H, including s: 1.74, CH_3), 1.13–1.00 (m, 5H, including s: 1.04, CH_3), 1.00–0.88 (m, 4H), 0.84, 0.82 (2 s, 6H, CH_3), 0.75 (s, 3H, CH_3), 0.70 (d, ^3J = 11.1 Hz, 1H); ^{13}C NMR (126 MHz, [D_4] MeOD): δ = 173.58, 169.46, 152.00, 110.27, 101.61, 79.66, 56.86, 52.00, 51.34, 50.99, 48.31, 43.85, 42.04, 40.79, 40.07, 39.94, 38.93, 38.32 (2C), 35.58, 35.43, 31.34, 29.76, 28.60, 28.04, 26.74, 22.08, 19.70, 19.41, 16.70, 16.65, 16.10, 15.31; IR (neat): 3565, 3495, 3355, 2940, 2925, 2865, 1590, 1450, 1385, 1190, 1045, 910, 870, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{52}N_2O_2+H]^+$ 509.4102; found 509.4101.

Method IV: Conventional preparation of isoxazoles 14a-i

To a solution of 20,29-dihydro betulin nitrile oxide **12** (0.100 g, 0.219 mmol, 1 equiv.) in CH_2Cl_2 (1.5 mL) under N_2 atmosphere alkyne (0.439 mmol, 2 equiv.) and Et_3N (0.091 mL, 0.657 mmol, 3 equiv.) were added and the reaction was stirred for 24 h. The residue was dissolved in EtOAc (40 mL), washed with brine (5× 10 mL), dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography on silica.

(17S)-17-(5-Hydroxymethyl-isoxazol-3-yl)-28-norlup-3β-ol 14a: 1) According to method I compound **14a** was prepared from 20,29-dihydro betulin oxime **10** (50 mg, 0.109 mmol, 1 equiv.), MTBS (50 mg, 0.160 mmol) and prop-2-yn-1-ol (32 μL, 0.550 mmol, 5 equiv.). Yield 45 mg, 80%.

2) According to method IV compound **14a** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.100 g, 0.219 mmol, 1 equiv.), prop-2-yn-1-ol (25 μL, 0.439 mmol, 2 equiv.), Et_3N (0.091 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (1.5 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc). Yield 101 mg, 90%. Colorless powder.

R_f = 0.52 (CH_2Cl_2/EtOAc 5:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.09 (s, 1H), 4.75 (s, 2H), 3.18 (dd, ^3J = 11.5, 4.8 Hz; 1H), 2.43 (td, ^3J = 12.2, 3.7 Hz; 1H), 2.30–2.22 (m, 1H), 2.10 (dt, ^2J = 13.1 Hz, ^3J = 3.3 Hz; 1H), 1.86 (septd, ^3J = 6.9, 2.6 Hz; 1H), 1.74–1.12 (m, 19H), 1.04 (dt, ^2J = 13.7 Hz, ^3J = 3.1 Hz; 1H), 1.00–0.60 (m, 23H; including 5 s: 0.97, 0.95, 0.79, 0.75, 0.74 CH_3 and 0.86 (d, ^3J = 6.9 Hz, CH_3)); ^{13}C NMR

(126 MHz, $CDCl_3$): δ = 169.82, 168.29, 101.48, 79.18, 56.88, 55.45, 50.45, 49.41, 44.29, 43.05, 40.93, 40.16, 38.99, 38.83, 37.48, 37.30, 34.52 (2C), 30.02, 28.73, 28.12, 27.51, 26.95, 23.16, 22.49, 22.33, 21.04, 18.42, 16.17 (2C), 15.51, 15.04, 14.99; IR (neat): 3330, 2940, 2865, 1605, 1445, 1365, 1190, 1035, 1000, 945, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{53}NO_3+H]^+$ 512.4098; found 512.4088.

(17S)-17-(5-(2-Hydroxyeth-1-yl)-isoxazol-3-yl)-28-norlup-3β-ol 14b: According to method IV compound **14b** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.100 g, 0.219 mmol, 1 equiv.), but-3-yn-1-ol (33 μL, 0.439 mmol, 2 equiv.), Et_3N (0.091 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (1.5 mL). Purified by column chromatography on silica with Hex/EtOAc (10%→50% EtOAc). Yield 67 mg, 58%. Colorless amorphous compound. R_f = 0.53 (Hex/EtOAc 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 5.93 (s, 1H), 3.95 (t, ^3J = 6.3 Hz, 2H), 3.18 (dd, ^3J = 11.4, 4.8 Hz; 1H), 3.00 (t, ^3J = 6.2 Hz; 2H), 2.44 (td, ^3J = 12.2, 3.7 Hz; 1H), 2.31–2.22 (m, 1H), 2.09 (dt, ^2J = 13.0 Hz, ^3J = 3.2 Hz; 1H), 1.86 (septd, ^3J = 6.9, 2.3 Hz; 1H), 1.75–1.14 (m, 19H), 1.07–0.63 (m, 24H; including 5 s: 0.97, 0.95, 0.79, 0.75, 0.74 CH_3 and 0.86 (d, ^3J = 6.8 Hz, CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.80, 168.34, 101.61, 79.16, 60.49, 55.44, 50.44, 50.28, 49.48, 44.34, 43.02, 40.93, 40.12, 38.99, 38.82, 37.44, 37.30, 34.53 (2C), 30.44, 30.03, 28.75, 28.12, 27.52, 26.94, 23.18, 22.55, 21.04, 18.41, 16.17, 16.09, 15.51, 15.05, 14.98; IR (neat): 3590, 3335, 2945, 2865, 1610, 1445, 1385, 1075, 1050, 1005, 985, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{34}H_{55}NO_3+H]^+$ 526.4255; found 526.4269.

(17S)-17-(5-(1-Hydroxycyclohexyl)-isoxazol-3-yl)-28-norlup-3β-ol 14c: According to method IV compound **14c** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.1 g, 0.219 mmol, 1 equiv.), 1-ethynylcyclohexan-1-ol (0.054 g, 0.439 mmol, 2 equiv.), Et_3N (0.091 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (2 mL). Purified by column chromatography on silica with CH_2Cl_2/EtOAc (0%→10% EtOAc). Yield 88 mg, 69%. Colorless amorphous compound. R_f = 0.46 (CH_2Cl_2/EtOAc 4:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.00 (s, 1H), 3.18 (dd, ^3J = 11.4, 4.6 Hz; 1H), 2.38 (td, ^3J = 12.1, 3.5 Hz; 1H), 2.31–2.23 (m, 1H), 2.11 (dt, ^2J = 13.0 Hz, ^3J = 3.1 Hz; 1H), 2.03–1.93 (m, 3H), 1.89–1.84 (m, 3H), 1.79–1.08 (m, 24H), 1.03 (dt, ^2J = 13.5 Hz, ^3J = 3.0 Hz; 1H) 1.08–0.62 (m, 23H; including 5 s: 0.97, 0.95, 0.79, 0.73, 0.72 CH_3 and 0.87 (d, ^3J = 6.8 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 176.31, 168.01, 99.24, 79.15, 70.54, 55.45, 50.43, 50.32, 49.60, 44.41, 43.01, 40.92, 40.02, 38.99, 38.82, 37.48, 37.30, 36.90, 36.86, 34.53, 34.49, 30.05, 28.75, 28.12, 27.54, 26.91, 25.32, 23.19, 22.56, 21.92, 21.90, 21.04, 18.41, 16.16, 15.95, 15.50, 15.05, 15.01; IR (neat): 3290, 2930, 2865, 1580, 1445, 1045, 990, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{38}H_{61}NO_3+H]^+$ 580.4724; found 580.4709.

(17S)-17-(5-(2-Hydroxypropan-2-yl)-isoxazol-3-yl)-28-norlup-3β-ol 14d: According to method IV compound **14d** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.1 g, 0.219 mmol, 1 equiv.), 2-methylbut-3-yn-2-ol (40 μL, 0.439 mmol, 2 equiv.), Et_3N (0.091 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (1.5 mL). Purified by column chromatography on silica with CH_2Cl_2/EtOAc (0%→10% EtOAc). Yield 88 mg, 74%. Colorless amorphous compound. R_f = 0.47 (CH_2Cl_2/EtOAc 4:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 5.98 (s, 1H), 3.18 (dd, ^3J = 10.7, 5.2 Hz; 1H), 2.42 (td, ^3J = 11.9, 3.6 Hz; 1H), 2.34–2.19 (m, 1H), 2.14–2.05 (m, 1H), 1.86 (septd, ^3J = 6.9, 2.8 Hz; 1H), 1.77–1.11 (m, 25H; including s: 1.62, 6H, CH_3), 1.10–0.60 (m, 24H; including 4 s: 0.97, 0.95, 0.79, 0.74 CH_3 and 0.87 (d, ^3J = 6.8 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 176.42, 168.12, 98.62, 79.17, 69.43, 55.47, 55.43, 50.46, 50.38, 49.57, 44.38, 43.03, 40.94, 40.05, 39.01, 38.85, 37.48, 37.32, 34.53, 30.04, 29.29, 29.26, 28.77, 28.14, 27.56, 26.94, 23.18, 22.56, 21.05, 18.42, 16.17, 16.00, 15.51, 15.06, 15.01; IR (neat): 3310, 2940, 2865, 1585, 1450, 1380, 1260, 1190, 1045, 970, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{57}NO_3+H]^+$ 540.4411; found 540.4380.

(17S)-17-(5-((2-Hydroxyethoxy)methyl)-isoxazol-3-yl)-28-norlup-3β-ol 14e: According to method IV compound **14e** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.1 g, 0.219 mmol, 1 equiv.), 2-(prop-2-yn-1-yloxy) ethan-1-ol (90 μL, 0.887 mmol, 4 equiv.), Et_3N (0.122 mL, 0.887 mmol, 4 equiv.), CH_2Cl_2 (1.5 mL). Purified by column chromatography on silica with CH_2Cl_2/EtOAc (0%→10% EtOAc). Yield 82 mg, 67%. Colorless amorphous compound. R_f = 0.39 (CH_2Cl_2/EtOAc 5:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.10 (s, 1H), 4.63 (s, 2H), 3.77 (t, ^3J = 3.9 Hz; 2H), 3.65 (t, ^3J = 4.0 Hz; 2H), 3.18 (dd, ^3J = 11.5, 4.7 Hz; 1H), 2.43 (td, ^3J = 12.0, 3.3 Hz; 1H), 2.30–2.24 (m, 1H), 2.09 (dt, ^2J = 13.0 Hz, ^3J = 3.1 Hz; 1H), 1.86 (septd, ^3J = 6.8, 2.8 Hz; 1H), 1.73–1.07 (m, 19H), 1.07–0.63 (m, 24H; including 5 s: 0.97, 0.95, 0.79, 0.74, 0.73 CH_3 and 0.87 (d, ^3J = 6.9 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.25, 167.51, 102.66, 79.15, 72.34, 64.23, 61.92, 55.46, 50.45, 50.40, 49.44, 44.30, 43.05, 40.94, 40.13, 39.00, 38.84, 37.47, 37.31, 34.53 (2C), 30.03, 28.74, 28.13, 27.54, 26.95, 23.17, 22.51, 21.04, 18.43, 16.18, 16.10, 15.51, 15.05, 15.00; IR (neat): 3450, 2925, 2865, 1725, 1610, 1455, 1385, 1365, 1265, 1045, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{57}NO_4+H]^+$ 556.4360; found 556.4386.

(17S)-17-(5-((Acetylthio)methyl)-isoxazol-3-yl)-28-norlup-3β-ol 14f: According to method IV compound **14f** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.1 g, 0.219 mmol, 1 equiv.), *S*-(prop-2-yn-1-yl) ethanethioate (45 μL, 0.4439 mmol, 2 equiv.), Et_3N (0.091 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (1.5 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→12% EtOAc). Yield 97 mg, 77%. Colorless amorphous compound. R_f = 0.71 (Hex/EtOAc 3:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 5.99 (s, 1H), 4.19 (d, ^2J = 15.1 Hz; 1H), 4.17 (d, ^2J = 15.1 Hz; 1H), 3.18 (dd, ^3J = 11.4, 4.7 Hz; 1H), 2.42–2.34 (m, 4H, including s 2.38 Ac), 2.29–2.21 (m, 1H), 2.06 (dt, ^2J = 13.2 Hz, ^3J = 3.3 Hz; 1H), 1.85 (septd, ^3J = 6.6, 2.8 Hz; 1H), 1.74–1.12 (m, 19H), 1.10–0.59 (m, 24H; including 5 s: 0.96, 0.95, 0.79, 0.74, 0.73 CH_3 and 0.86 (d, ^3J = 6.9 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 193.91, 168.53, 166.78, 102.45, 79.14, 55.46, 50.45, 50.35, 49.47, 44.28, 43.01, 40.93, 40.05, 38.99, 38.83, 37.43, 37.31, 34.51, 34.47, 30.40, 30.02, 28.71, 28.12, 27.54, 26.93, 23.96, 23.16, 22.50, 21.04, 18.42, 16.17, 16.05, 15.51, 15.04, 14.99; IR (neat): 3610, 2940, 2865, 1685, 1605, 1455, 1405, 1385, 1360, 1045, 1005, 640 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{55}NO_3S+H]^+$ 570.3975; found 570.3986.

(17S)-17-(5-(2,3,4,6-Tetra-*O*-acetyl-β-D–gluco-pyranosyloxymethyl)-isoxazol-3-yl)-28-norlup-3β-ol 14g: According to method IV compound **14g** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.150 g, 0.329 mmol, 1 equiv.), propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (235 mg, 0.606 mmol, 2 equiv.), Et_3N (0.127 mL, 0.914 mmol, 3 equiv.), CH_2Cl_2 (2 mL). Purified by column chromatography on silica with CH_2Cl_2/EtOAc (0%→10% EtOAc). Yield 155 mg, 56%. Colorless amorphous compound. R_f = 0.46 (CH_2Cl_2/EtOAc 4:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.09 (s, 1H), 5.20 (dd, ^3J = 9.5, 9.4 Hz; 1H), 5.10 (dd, ^3J = 9.8, 9.6 Hz; 1H), 5.04 (dd, ^3J = 9.5, 8.0 Hz; 1H), 4.86 (d, ^2J = 13.8 Hz; 1H), 4.74 (d, ^2J = 13.8 Hz; 1H), 4.60 (d, ^3J = 7.9 Hz; 1H), 4.28 (dd, ^2J = 12.4 Hz, ^3J = 4.6 Hz; 1H), 4.15 (dd, ^2J = 12.4 Hz, ^3J = 2.3 Hz; 1H), 3.71 (ddd, ^3J = 10.0, 4.6, 2.3 Hz; 1H), 3.18 (dd, ^3J = 11.4, 4.7 Hz; 1H), 2.39 (td, ^3J = 12.2, 3.6 Hz; 1H), 2.28–2.20 (m, 1H), 2.15–1.95 (m, 13H; including 3 s: 2.09, 2.02, 2.00 Ac), 1.86 (septd, ^3J = 6.8, 2.7 Hz; 1H), 1.72–1.12 (m, 19H), 1.05 (dt, ^2J = 13.6 Hz, ^3J = 3.1 Hz; 1H), 1.02–0.64 (m, 23H; including 5 s: 0.97, 0.95, 0.80, 0.76, 0.74 CH_3 and 0.86 (d, ^3J = 6.9 Hz, CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.76, 170.29, 169.53, 169.47, 168.29, 166.42, 103.24, 99.92, 79.11, 72.77, 72.18, 71.16, 68.37, 61.90, 61.78, 55.44, 50.41 (2C), 49.40, 44.32, 43.04, 40.94, 40.13, 38.99, 38.82, 37.52, 37.30, 34.50, 34.45, 30.02, 28.69, 28.12, 27.52, 26.92, 23.15, 22.49, 21.03, 20.88, 20.76, 20.74, 20.71, 18.38, 16.22, 16.15, 15.49, 15.02, 14.98; IR (neat): 3595, 2945, 2865, 1750, 1450, 1230, 1035, 815,

600 cm^{-1}; HRMS (ESI): m/z calcd. for $[C_{47}H_{71}NO_{12}+H]^+$ 842.5049; found 842.5023.

(17S)-17-(5-(2,3,4,6-Tetra-*O*-acetyl-α-D–manno-pyranosyloxymethyl)-isoxazol-3-yl)-28-norlup-3β-ol 14h: According to method IV compound **14h** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.100 g, 0.219 mmol, 1 equiv.), propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (169 mg, 0.439 mmol, 2 equiv.), Et_3N (0.095 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (2 mL). Purified by column chromatography on silica with CH_2Cl_2/EtOAc (0%→10% EtOAc). Yield 89 mg, 48%. Colorless amorphous compound. R_f = 0.56 (CH_2Cl_2/EtOAc 5:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.13 (s, 1H), 5.37–5.28 (m, 3H), 4.94 (d, ^3J = 1.4 Hz; 1H), 4.74 (d, ^2J = 13.5 Hz; 1H), 4.66 (d, ^2J = 13.5 Hz; 1H), 4.31 (dd, ^2J = 12.3 Hz, ^3J = 5.0 Hz; 1H), 4.09 (dd, ^2J = 12.3 Hz, ^3J = 2.3 Hz; 1H), 4.04 (ddd, ^3J = 9.0, 4.8, 2.3 Hz; 1H), 3.18 (dd, ^3J = 11.4, 4.7 Hz; 1H), 2.43 (td, ^3J = 12.0, 3.4 Hz; 1H), 2.30–2.23 (m, 1H), 2.21–1.94 (m, 13H; including 4 s: 2.15, 2.11, 2.04, 1.99 Ac), 1.93–1.82 (m, 1H), 1.75–1.09 (m, 19H), 1.08–0.60 (m, 24H; including 5 s: 0.98, 0.95, 0.79, 0.75, 0.73 CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.75, 170.04, 170.02, 169.81, 168.36, 165.91, 103.36, 97.52, 79.13, 69.41, 69.13, 69.05, 66.09, 62.37, 60.47, 55.45, 50.45, 49.42, 44.26, 43.05, 40.94, 40.08, 38.99, 38.83, 37.46, 37.31, 34.53, 34.48, 31.73, 30.01, 28.75, 28.12, 27.54, 26.93, 23.15, 22.50, 21.03, 20.99, 20.89, 20.83, 20.81, 18.41, 16.17, 16.12, 15.50, 15.04, 14.98; IR (neat): 2945, 2865, 1745, 1450, 1365, 1220, 1045, 980, 800, 600 cm^{-1}; HRMS (ESI): m/z calcd. for $[C_{47}H_{71}NO_{12}+H]^+$ 842.5049; found 842.5027.

(17S)-7-(5-((1,3-Dioxoisoindolin-2-yl)methyl)-isoxazol-3-yl)-28-norlup-3β-ol 14i: According to method IV compound **14i** was prepared from 20,29-dihydro betulin nitrile oxide **12** (0.100 g, 0.219 mmol, 1 equiv.), 2-(prop-2-yn-1-yl) isoindoline-1,3-dione (75 mg, 0.432 mmol, 2 equiv.), Et_3N (0.091 mL, 0.657 mmol, 3 equiv.), CH_2Cl_2 (2 mL). Purified by column chromatography on silica with Hex/EtOAc (0%→17% EtOAc). Yield 85 mg, 61%. Colorless amorphous compound. R_f = 0.43 (Hex/EtOAc 2:1);^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.89 (dd, ^3J = 5.4, 3.1 Hz; 2H), 7.75 (dd, ^3J = 5.5, 3.0 Hz; 2H), 6.08 (s, 1H), 4.96 (2d, ^2J = 16.6 Hz; 2H), 3.17 (dd, ^3J = 11.4, 4.8 Hz; 1H), 2.39 (td, ^3J = 12.3, 3.7 Hz; 1H), 2.27–2.20 (m, 1H), 2.07–2.00 (m, 1H), 1.84 (septd, ^3J = 6.7, 2.9 Hz; 1H), 1.72–1.11 (m, 19H), 1.06–0.62 (m, 24H; including 4 s: 0.95, 0.77, 0.74, 0.70 CH_3 and 0.84 (d, ^3J = 6.9 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.46, 167.36, 164.88, 134.43 (2C), 132.08 (2C), 123.80 (2C), 102.56, 79.15, 55.45, 50.44, 50.36, 49.44, 44.21, 43.00, 40.91, 39.99, 38.99, 38.82, 37.39, 37.29, 34.49, 34.46, 33.37, 29.98, 29.96, 28.69, 28.12, 27.54, 26.92, 23.14, 22.46, 21.01, 18.41, 16.17, 16.06, 15.50, 15.03, 14.95; IR (neat): 3485, 2925, 2865, 1775, 1720, 1610, 1465, 1390, 945, 710, 530 cm^{-1}; HRMS (ESI): m/z calcd. for $[C_{41}H_{56}N_2O_4+H]^+$ 641.4313; found 641.4328.

(17S)-17-(5-(Mercaptomethyl)-isoxazol-3-yl)-28-norlup-3β-ol 14f': To a solution of compound **14f** (0.065 g, 0.114 mmol, 1 equiv.) in MeOH (5 mL) NaOMe (2 mg, 0.046 mmol, 0.4 equiv.) was added. Reaction was stirred for 12 h at room temperature, the reaction was quenched by addition of saturated aq. solution of NH4Cl (2.5 mL), further ethyl acetate (40 mL) was added to the reaction; the mixture was washed with brine (4×10 mL), dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography (25% Hex/EtOAc). Yield 43 mg, 72%. Colorless amorphous compound. R_f = 0.48 (Hex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.06 (s, 1H), 3.81 (2d, ^2J = 15.1 Hz; 2H), 3.18 (dd, ^3J = 11.4, 4.6 Hz; 1H), 2.45 (td, ^3J = 12.1, 3.5 Hz; 1H), 2.33–2.23 (m, 1H), 2.09 (dt, ^2J = 13.1 Hz, ^3J = 3.0 Hz; 1H), 1.86 (septd, ^3J = 6.8, 2.8 Hz; 1H), 1.77–1.12 (m, 19H), 1.05 (dt, ^2J = 13.5 Hz, ^3J = 2.9 Hz; 1H), 1.01–0.62 (m, 23H; including 5 s: 0.97, 0.95, 0.78, 0.74, 0.73 CH_3 and 0.86 (d, ^3J = 6.8 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.64, 166.33, 103.43, 79.14, 55.45, 50.46, 50.45, 49.37, 44.25, 43.06, 40.94, 40.20, 38.99, 38.83, 37.48, 37.31, 34.57, 34.51, 33.57, 30.02, 28.75, 28.12, 27.53, 26.97, 23.17, 22.55, 21.07, 18.42, 16.22 (2C), 15.50, 15.05, 14.99; IR (neat): 3465, 2930, 2865, 1730, 1600, 1455, 1385, 1365, 1245, 1035, 1005, 800, 730 cm^{-1}; HRMS (ESI): m/z calcd. for $[C_{33}H_{53}NO_2S+H]^+$ 528.3870; found 528.3879.

(17S)-17-(5-(β-D-Gluco-pyranosyloxymethyl)-isoxazol-3-yl)-28-norlup-3β-ol 14g': To a solution of compound **14g** (41 mg, 0.049 mmol, 1 equiv.) in MeOH (5 mL) NaOMe (1 mg, 0.024 mmol, 0.5 equiv.) was added. Reaction was stirred for 12 h at room temperature; the reaction was quenched by addition of ion-exchange resin (Dowex 50WX8, 50–100 mesh, H− form) till pH 7, filtered and evaporated in vacuum. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→16% EtOAc). Yield 24 mg, 73%. Colorless amorphous compound. R_f = 0.31 (Hex/EtOAc 9:1); ^{1}H NMR (500 MHz, [D_8]THF): δ = 6.32 (s, 1H), 4.89 (d, ^2J = 13.5 Hz; 1H), 4.71 (d, ^2J = 13.5 Hz; 1H), 4.59 (d, ^3J = 3.4 Hz; 1H), 4.46 (s, 1H), 4.36 (d, ^3J = 1.7 Hz; 1H), 4.29 (d, ^3J = 7.7 Hz; 1H), 3.82–3.75 (m, 1H), 3.63–3.54 (m, 2H), 3.34 (d, ^3J = 5.0 Hz; 1H), 3.22 (m, 3H), 3.12 (td, ^3J = 8.0, 2.9 Hz; 1H), 3.03 (dt, ^3J = 10.9, 5.0 Hz; 1H), 2.57 (td, ^3J = 12.1, 3.9 Hz; 1H), 2.37–2.31 (m, 1H), 2.14 (dt, ^2J = 12.6 Hz, ^3J = 3.0 Hz; 1H), 1.89 (septd, ^3J = 6.8, 2.8 Hz; 1H), 1.70–1.21 (m, 19H), 1.06–1.00 (m, 4H, including s: 1.01 CH_3), 0.96–0.66 (m, 20H; including 4 s: 0.93, 0.83, 0.79, 0.72 CH_3 and 0.88 (d, ^3J = 6.9 Hz, CH_3)); ^{13}C NMR (126 MHz, [D_8]THF): δ = 168.97, 168.47, 103.80, 103.27, 78.54, 78.26, 78.13, 74.98, 71.55, 63.19, 62.19, 56.57, 51.47, 51.08, 50.38, 45.13, 43.79, 41.83, 40.86, 39.80, 38.29, 38.14, 35.50, 35.04, 30.92, 30.69, 29.56, 28.61, 28.54, 27.95, 23.45, 23.13, 21.92, 19.30, 16.72, 16.67, 16.12, 15.29, 15.23; IR (neat): 3335, 2925, 2865, 1610, 1450, 1075, 1030, 815 cm^{-1}; HRMS (ESI): m/z calcd. for $[C_{39}H_{63}NO_8+H]^+$ 674.4626; found 674.4620.

(17S)-17-(5-(α-D-Manno-pyranosyloxymethyl)-isoxazol-3-yl)-28-norlup-3β-ol 14h': To a solution of compound **14h** (70 mg, 0.083 mmol, 1 equiv.) in MeOH (5 mL) NaOMe (3 mg, 0.055 mmol, 0.5 equiv.) was added. Reaction was stirred for 12 h at room temperature; the reaction was quenched by addition of ion-exchange resin (Dowex 50WX8, 50–100 mesh, H− form) till pH 7, filtered and evaporated in vacuum. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→16% EtOAc). Yield 43 mg, 77%. Colorless amorphous compound. R_f = 0.31 (Hex/EtOAc 9:1); ^{1}H NMR (500 MHz, [D_8]THF): δ = 6.25 (s, 1H), 4.82 (d, ^3J = 1.0 Hz; 1H), 4.72 (d, ^2J = 13.5 Hz; 1H), 4.61 (d, ^2J = 13.5 Hz; 1H), 4.14 (d, ^3J = 3.1 Hz; 1H), 4.08 (d, ^3J = 3.6 Hz; 1H), 4.02 (d, ^3J = 3.0 Hz; 1H), 3.76–3.72 (m, 2H), 3.65–3.53 (m, 4H), 3.48–3.43 (m, 1H), 3.32 (d, ^3J = 5.3 Hz; 1H), 3.03 (dt, ^3J = 10.8, 5.3 Hz; 1H), 2.59 (td, ^3J = 12.3, 3.7 Hz; 1H), 2.38–2.31 (m, 1H), 2.13 (dt, ^2J = 12.8 Hz, ^3J = 3.1 Hz; 1H), 1.89 (septd, ^3J = 6.9, 2.8 Hz; 1H), 1.70–1.21 (m, 19H), 1.07–0.66 (m, 24H; including 5 s: 1.00, 0.93, 0.83, 0.78, 0.71 CH_3 and 0.88 (d, ^3J = 6.9 Hz, CH_3)); ^{13}C NMR (126 MHz, [D_8]THF): δ = 168.72, 168.49, 103.19, 101.09, 78.54, 74.95, 72.74, 71.70, 68.92, 63.20, 59.99, 56.58, 51.49, 51.06, 50.42, 45.15, 43.77, 41.82, 40.77, 39.80, 38.22, 38.14, 35.49, 35.06, 30.94, 30.69, 29.56, 28.61, 28.55, 27.96, 23.45, 23.14, 21.92, 19.30, 16.67, 16.60, 16.12, 15.30, 15.22; IR (neat): 3335, 2925, 2865, 1610, 1450, 1385, 1365, 1030, 970, 800 cm^{-1}; HRMS (ESI): m/z calcd. for $[C_{39}H_{63}NO_8+H]^+$ 674.4626; found 674.4639.

(17S)-17-(5-(Aminomethyl)-isoxazol-3-yl)-28-norlup-3β-ol 14i': $NH_2NH_2 \cdot H_2O$ (21 μL, 0.663 mmol, 5 equiv.) is added to solution of **14i** (0.085 g, 0.132 mmol, 1 equiv.) in EtOH (10 mL) reaction is heated to reflux for 2 h. After cooling, saturated NH_4Cl solution (0.2 mL) is added, reaction mixture is evaporated and purified by column chromatography on silica with CH_2Cl_2/MeOH (0%→2% MeOH). Yield 41 mg, 60%. Colorless amorphous powder. R_f = 0.43 (CH_2Cl_2/MeOH 19:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.97 (s, 1H), 3.95 (s, 2H), 3.18 (dd, ^3J = 11.4, 4.7 Hz; 1H), 2.44 (td, ^3J = 12.3, 3.5 Hz; 1H), 2.30–2.22 (m, 1H), 2.08 (dt, ^2J = 13.2 Hz, ^3J = 3.2 Hz; 1H), 2.01 (b.s., 2H), 1.86 (septd, ^3J = 6.9, 2.8 Hz; 1H), 1.72–1.14 (m, 19H), 1.08–0.60 (m, 24H; including 5 s: 0.97, 0.95, 0.79, 0.76, 0.74 CH_3 and 0.86 (d,

^3J = 6.8 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 172.07, 168.25, 100.10, 79.14, 55.47, 50.46, 50.39, 49.44, 44.30, 43.05, 40.95, 40.16, 38.99, 38.84, 38.35, 37.46, 37.32, 34.55 (2C), 30.02, 28.75, 28.13, 27.54, 26.96, 23.15, 22.52, 21.05, 18.43, 16.17 (2C), 15.51, 15.05, 14.99; IR (neat): 3365, 2925, 2865, 1605, 1455, 1385, 1365, 1045, 1005, 985, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{54}N_2O_2+H]^+$ 511.4258; found 511.4270.

(17S)-17-(5-(Formyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol 15: MnO_2 (375 mg, 4.316 mmol, 20 equiv.) is added to solution of **13a** (110 mg, 0.216 mmol, 1 equiv.) in $CHCl_3$ (8 mL) reaction is heated to reflux for 6 h. After cooling, reaction mixture is filtered through celite pad and evaporated. Yield 103 mg, 94%. Colorless amorphous powder. ^1H NMR (500 MHz, $CDCl_3$): δ = 9.98 (s, 1H), 6.89 (s, 1H), 4.77 (s, 1H), 4.63 (s, 1H), 3.17 (dd, ^3J = 11.4, 4.7 Hz; 1H), 3.05 (td, ^3J = 10.9, 4.5 Hz; 1H), 2.40 (td, ^3J = 12.5, 3.4 Hz; 1H), 2.08 (dt, ^2J = 13.5 Hz, ^3J = 3.5 Hz; 1H), 1.85 (t, ^3J = 11.5 Hz; 1H), 1.79–1.16 (m, 21H, including s: 1.73 CH_3), 1.16–0.84 (m, 8H, including 2 s: 1.01, 0.95 CH_3), 0.79, 0.76, 0.73 (3 s, 9H, CH_3), 0.64 (d, ^3J = 9.3 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.82, 169.14, 165.14, 150.35, 110.07, 108.77, 79.11, 55.48, 50.67, 50.40, 49.85, 46.93, 42.91, 40.93, 39.91, 38.99, 38.84, 37.79, 37.33, 34.55, 34.44, 30.23, 28.61, 28.11, 27.54, 25.52, 20.98, 19.67, 18.40, 16.23, 16.08, 15.48, 15.08; IR (neat): 3535, 2925, 2865, 1690, 1455, 1390, 1245, 1045, 885, 755; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{49}NO_3+H]^+$ 508.3785; found 508.3788.

(17S)-17-(5-(Formyl)-isoxazol-3-yl)-28-norlup-3β-ol 16: MnO_2 (340 mg, 3.908 mmol, 20 equiv.) is added to solution of **14a** (100 mg, 0.195 mmol, 1 equiv.) in $CHCl_3$ (8 mL) reaction is heated to reflux for 20 h. After cooling, reaction mixture is filtered through celite pad and evaporated. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→16% EtOAc). Yield 80 mg, 81%. Colorless amorphous compound. R_f = 0.47 (EtOAc/Hex = 1/5). ^1H NMR (500 MHz, $CDCl_3$): δ = 9.97 (s, 1H), 6.87 (s, 1H), 3.18 (dd, ^3J = 11.5, 4.7 Hz; 1H), 2.34 (td, ^3J = 11.9, 3.6 Hz; 1H), 2.31–2.24 (m, 1H), 2.16 (dt, ^2J = 13.2 Hz, ^3J = 3.1 Hz; 1H), 1.86 (septd, ^3J = 6.9, 2.7 Hz; 1H), 1.75–1.17 (m, 19H), 1.11–1.06 (m, 1H), 1.01–0.64 (m, 23H; including 5 s: 0.99, 0.95, 0.79, 0.74, 0.72 CH_3, 0.86 (d, ^3J = 6.8 Hz; CH_3) and 0.81 (d, ^3J = 6.7 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.90, 169.33, 165.09, 108.89, 79.13, 55.45, 50.72, 50.40, 49.38, 44.32, 43.13, 40.93, 40.13, 39.00, 38.84, 37.68, 37.30, 34.50, 34.37, 30.03, 28.61, 28.13, 27.53, 26.93, 23.15, 22.39, 21.00, 18.40, 16.18, 16.08, 15.50, 15.05, 15.01; IR (neat): 3515, 2940, 2865, 2820, 1695, 1455, 1385, 1275, 1245, 1045, 835, 755 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{51}NO_3+H]^+$ 510.3942; found 510.3962. Additionally ketone **16a** as a byproduct was isolated: (4 mg, 5%) as colorless amorphous compound. R_f = 0.31 (EtOAc/Hex = 1/5). ^1H NMR (500 MHz, $CDCl_3$): δ = 9.98 (s, 1H), 6.88 (s, 1H), 2.54–2.44 (m, 1H), 2.39 (m, 2H), 2.33–2.23 (m, 1H), 2.15 (dt, ^2J = 13.4 Hz, ^3J = 3.8 Hz; 1H), 1.95–1.82 (m, 3H), 1.77–1.66 (m, 3H), 1.65–1.18 (m, 12H), 1.16–0.67 (m, 24H; including 4 s: 1.06, 1.00 (6H), 0.90, 0.76 CH_3, 0.88 (d, ^3J = 6.7 Hz; CH_3) and 0.81 (d, ^3J = 6.7 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 218.16, 178.86, 169.28, 165.11, 108.84, 55.12, 50.70, 49.76, 49.30, 47.48, 44.26, 43.17, 40.87, 40.09, 39.72, 37.74, 37.01, 34.31, 34.28, 33.81, 30.02, 28.59, 26.92, 26.70, 23.14, 22.38, 21.51, 21.21, 19.72, 15.98, 15.91, 15.00, 14.96; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{51}NO_3+H]^+$ 508,3785; found 508.3775.

(17S)-17-(5-(Acetyloxymethyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol acetate 19: Ac_2O (0.320 mL, 3.374 mmol, 4 equiv.) is slowly added to cooled (0 °C) solution of **13a** (0.430 g, 0.843 mmol, 1 equiv.) and DMAP (0.02 g, 0.136 mmol, 0.2 equiv.) in Py (5 mL) under inert atmosphere. After stirring for 18 h at room temperature, most of the Py was removed under reduce pressure. The residue was dissolved in EtOAc (25 mL), washed with 2% HCl aq. solution (10×5 mL), saturated aq. solution $NaHCO_3$ (2×3 mL) and brine (5×3 mL), dried over Na_2SO_4, filtered, and evaporated in vacuum. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc) giving 488 mg (97%) of diacetate analog **19** as colorless solid. R_f = 0.61 (Hex/EtOAc 3:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.14 (s, 1H), 5.17 (d, ^2J = 14.3 Hz; 1H), 5.14 (d, ^2J = 14.3 Hz; 1H), 4.76 (s, 1H), 4.61 (s, 1H), 4.46 (dd, ^3J = 10.5, 5.8 Hz; 1H), 3.05 (td, ^3J = 11.0, 4.5 Hz; 1H), 2.47 (td, ^3J = 12.7, 3.4 Hz; 1H), 2.14 (s, 3H, Ac), 2.07–1.98 (m, 4H, including s: 2.03, Ac), 1.84–1.20 (m, 21H, including s: 1.72, CH_3), 1.11–0.92 (m, 6H, including s: 0.99, CH_3), 0.88–0.73 (m, 13H, including 4 s: 0.83, 0.82, 0.81, 0.80, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.16, 170.39, 168.23, 165.41, 150.78, 109.83, 103.48, 81.11, 56.71, 55.60, 50.65, 50.13, 49.94, 46.95, 42.86, 40.98, 39.88, 38.54, 37.94, 37.62, 37.28, 34.61, 34.43, 30.40, 28.69, 28.09, 25.52, 23.85, 21.46, 21.05, 20.85, 19.68, 18.32, 16.63, 16.30, 16.13, 15.01; IR (neat): 2930, 2870, 1755, 1725, 1645, 1450, 1360, 1240, 1225, 1020, 980, 875, 810 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{37}H_{55}NO_5+H]^+$ 594,4153; found 594,4157.

(17S)-17-(5-(Acetyloxymethyl)-isoxazol-3-yl)-28-norlup-3β-ol acetate 20: Ac_2O (0.794 mL, 8.400 mmol, 4.3 equiv.) is slowly added to cooled (0 °C) solution of **14a** (1.00 g, 1.954 mmol, 1 equiv.) and DMAP (0.047 g, 0.391 mmol, 0.2 equiv.) in Py (10 mL) under inert atmosphere. After stirring for 6 h at room temperature, most of the Py was removed under reduce pressure. The residue was dissolved in EtOAc (100 mL), washed with 2% HCl aq. solution (10×5 mL), saturated aq. solution $NaHCO_3$ (2×3 mL) and brine (5×3 mL), dried over Na_2SO_4, filtered, and evaporated in vacuum. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc) giving 1.038 g (90%) of diacetate analog **20** as a colorless amorphous compound. R_f = 0.53 (Hex/EtOAc 5:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.13 (s, 1H), 5.15 (s, 2H), 4.46 (dd, ^3J = 10.7, 5.6 Hz; 1H), 2.42 (td, ^3J = 12.1, 3.6 Hz; 1), 2.30–2.22 (m, 1H), 2.13 (s, 3H, Ac), 2.10–2.02 (m, 4H; including s: 2.03 Ac), 1.87 (septd, ^3J = 6.7, 2.7 Hz; 1H), 1.73–1.16 (m, 19H), 1.07–0.71 (m, 24H; including 5 s: 0.97, 0.83, 0.82, 0.81, 0.75 CH_3 and 0.86 (d, ^3J = 6.8 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.16, 170.39, 168.39, 165.25, 103.56, 81.09, 56.71, 55.54, 50.41, 50.36, 49.41, 44.25, 43.03, 40.95, 40.07, 38.51, 37.93, 37.43, 37.22, 34.47, 34.46, 30.00, 28.69, 28.08, 26.91, 23.83, 23.15, 22.47, 21.47, 21.05, 20.86, 18.30, 16.63, 16.24, 16.09, 15.04, 14.94; IR (neat): 2925, 2855, 1750, 1725, 1615, 1455, 1365, 1240, 1020, 980, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{37}H_{57}NO_5+H]^+$ 596.4310; found 596.4307.

3-{(17S)-[3β-Acetyloxy-28-norlupan-20(29)-en]-17-yl}-(isoxazol-5-yl) methanol 21: NaOMe (0.021 g, 0.395 mmol, 0.5 equiv.) is added to solution of **19** (0.470 g, 0.791 mmol, 1 equiv.) in MeOH (15 mL). Reaction was stirred for 2.5 h at room temperature, the reaction was quenched by addition of ion-exchange resin (Dowex 50WX8, 50–100 mesh, H − form) till pH 7, filtered and evaporated in vacuum. Yield 434 mg, 99%. White solid. ^1H NMR (500 MHz, $CDCl_3$): δ = 6.11 (s, 1H), 4.76 (s, 3H), 4.61 (s, 1H), 4.46 (dd, ^3J = 10.5, 5.9 Hz; 1H), 3.05 (td, ^3J = 11.1, 4.5 Hz; 1H), 2.48 (td, ^3J = 12.6, 3.6 Hz; 1H), 2.12–1.98 (m, 4H, including s: 2.03, Ac), 1.82–1.21 (m, 21H, including s: 1.72, CH_3), 1.11–0.92 (m, 6H, including s: 0.99, CH_3), 0.86–0.74 (m, 13H, including 4 s: 0.83, 0.82, 0.81, 0.80, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.21, 169.89, 168.12, 150.81, 109.80, 101.41, 81.15, 56.91, 55.59, 50.65, 50.16, 49.92, 46.97, 42.87, 40.98, 39.94, 38.53, 37.94, 37.64, 37.27, 34.65, 34.42, 30.42, 28.71, 28.08, 25.52, 23.84, 21.46, 21.05, 19.68, 18.32, 16.62, 16.29, 16.19, 15.01; IR (neat): 3400, 2940, 2865, 1730, 1710, 1610, 1450, 1370, 1245, 1195, 1025, 980, 880, 800, 750 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{53}NO_4+H]^+$ 552.4047; found 552.4066.

3-{(17S)-[3β-Acetyloxy-28-norlupane]-17-yl}-(isoxazol-5-yl) methanol 22: NaOMe (0.053 g, 0.978 mmol, 0.5 equiv.) is added to solution of **20** (1.166 g, 1.957 mmol, 1 equiv.) in MeOH (220 mL). Reaction was stirred for 2.5 h at room temperature, the reaction was quenched by saturated NH_4Cl solution (15 mL) is added. Reaction mixture was dissolved in EtOAc (250 mL), washed with H_2O (1×20 mL) and brine (5×15 mL), dried over Na_2SO_4, filtered,

and evaporated in vacuum. The residue was purified by column chromatography on silica with Hex/EtOAc (0%→10% EtOAc) giving 0.802 g (74%) of monoacetate analog **22** as a colorless amorphous compound. R_f = 0.29 (Hex/EtOAc 5:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.09 (s, 1H), 4.75 (s, 2H), 4.46 (dd, ^3J = 10.6, 5.7 Hz; 1H), 2.43 (td, ^3J = 12.1, 3.6 Hz; 1H), 2.29–2.23 (m, 1H), 2.22–.17 (m, 1H), 2.09 (dt, ^2J = 13.1 Hz, ^3J = 3.3 Hz; 1H), 2.03 (s, 3H, Ac), 1.86 (septd, ^3J = 6.9, 2.7 Hz; 1H,), 1.74–1.15 (m, 19H), 1.07–0.93 (m, 5H; including s: 0.97, CH_3), 0.89–0.73 (m, 19H; including 0.86 (d, ^3J = 6.9 Hz; CH_3) and 4 s: 0.82, 0.81 0.81, 0.75 CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.24, 169.83, 168.29, 101.46, 81.14, 56.88, 55.52, 50.43, 50.35, 49.39, 44.28, 43.04, 40.94, 40.14, 38.50, 37.92, 37.45, 37.21, 34.50, 34.45, 30.01, 28.70, 28.07, 26.91, 23.81, 23.15, 22.49, 21.46, 21.04, 18.30, 16.62, 16.23, 16.15, 15.04, 14.94; IR (neat): 2950, 2865, 1735, 1615, 1455, 1365, 1240, 1030, 980, 800 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{55}NO_4+H]^+$ 554.4204; found 554.4247.

(17S)-17-(5-(Formyl)-isoxazol-3-yl)-28-norlup-20(29)-en-3β-ol acetate 23: PCC (0.365 g, 1.692 mmol, 2 equiv.) is slowly added to solution of **21** (0.467 g, 0.846 mmol, 1 equiv.) in CH_2Cl_2 (20 mL). After stirring at room temperature for 2 h, reaction mixture is filtered through silica gel and evaporated under reduced pressure to give 374 mg (80%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.98 (s, 1H), 6.89 (s, 1H), 4.77 (s, 1H), 4.64 (s, 1H), 4.46 (dd, ^3J = 10.7, 5.6 Hz; 1H), 3.05 (td, ^3J = 10.9, 4.5 Hz; 1H), 2.41 (td, ^3J = 12.5, 3.5 Hz; 1H), 2.08 (dt, ^3J = 13.7, 3.1 Hz; 1H), 2.03 (s, 3H, Ac), 1.85 (t, ^3J = 11.5 Hz; 1H), 1.79–1.21 (m, 20H, including s: 1.73, CH_3), 1.14–0.92 (m, 6H, including s: 1.00, CH_3), 0.85–0.73 (m, 13H, including 4 s, 0.83, 0.82, 0.82, 0.76, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.80, 171.16, 169.17, 165.15, 150.33, 110.12, 108.77, 81.07, 55.59, 50.60, 50.42, 49.87, 46.97, 42.93, 40.97, 39.92, 38.54, 37.94, 37.80, 37.26, 34.56, 34.39, 30.24, 28.61, 28.08, 25.50, 23.84, 21.46, 21.01, 19.65, 18.30, 16.62, 16.30, 16.10, 15.05; IR (neat): 3390, 3290, 2940, 2870, 1705, 1645, 1450, 1370, 1245, 1060, 1025, 975, 885, 800, 750 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{51}NO_4+H]^+$ 550.3891; found 550.3894.

(17S)-17-(5-(Formyl)-isoxazol-3-yl)-28-norlup-3β-ol acetate 24: PCC (0.213 g, 0.988 mmol, 1.7 equiv.) is slowly added to mixture of **22** (0.322 g, 2.270 mmol, 1 equiv.) and Na_2SO_4 (300 mg) in CH_2Cl_2 (10 mL). After stirring at room temperature for 30 min, reaction mixture is filtered through silica gel and evaporated under reduced pressure to give 318 mg (99%) as a colorless amorphous compound. R_f = 0.39 (Hex/EtOAc 9:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.97 (s, 1H), 6.87 (s, 1H), 4.47 (dd, ^3J = 10.9, 5.5 Hz; 1H), 2.34 (td, ^3J = 11.8, 3.5 Hz; 1H), 2.30-2.24 (m, 1H), 2.15 (dt, ^2J = 13.2 Hz, ^3J = 3.2 Hz; 1H), 2.04 (s, 3H, Ac), 1.88 (septd, ^3J = 6.8, 2.9 Hz; 1H), 1.75–1.20 (m, 19H), 1.08 (dt, ^2J = 13.8 Hz, ^3J = 3.0 Hz; 1H), 1.02–0.93 (m, 4H; including s, 0.98 CH_3), 0.92–0.78 (m, 19H; including 0.88 (d, ^3J = 6.9 Hz; CH_3) and 4 s, 0.83, 0.82, 0.82, 0.72 CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.89, 171.18, 169.33, 165.08, 108.91, 81.06, 55.53, 50.72, 50.31, 49.37, 44.32, 43.12, 40.94, 40.12, 38.52, 37.93, 37.66, 37.22, 34.43, 34.36, 30.02, 28.60, 28.08, 26.90, 23.82, 23.15, 22.39, 21.47, 21.01, 18.28, 16.63, 16.24, 16.08, 15.01 (2C); IR (neat): 2945, 2870, 1730, 1705, 1585, 1455, 1365, 1245, 1025, 980, 760 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{53}NO_4+H]^+$ 552.4047; found 552.4036.

3-{(17S)-[3β-Acetyloxy-28-norlupan-20(29)-en]-17-yl}-(isoxazol-5-yl)carboxylic acid 25: A solution of $NaClO_2$ (0.595 g, 5.267 mmol, 8 equiv. (79% purity by iodometric titration)) and NaH_2PO_4 (0.553 g, 4.606 mmol, 7 equiv.) in 8 mL of water was added dropwise to a stirred mixture of aldehyde **23** (0.362 g, 0.658 mmol, 1 equiv.) and 2,3-dimethyl-2-butene (4 mL) in THF/t-BuOH (1:1, 24 mL). After stirring at room temperature for 3 h, CH_2Cl_2 (20 mL) and water (20 mL) was added to reaction mixture, reaction was extracted with CH_2Cl_2 (7×5 mL), combined organic phases were washed with water (3×20 mL), dried over Na_2SO_4, filtered, and evaporated in vacuum. The residue was purified by RP C18 silica column chromatography (50:50→95:5 MeOH/H_2O + 0.1% AcOH) giving 301 mg (82%) of **25** as a colorless amorphous compound. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.95 (s, 1H), 4.77 (s, 1H), 4.63 (s, 1H), 4.47 (dd, ^3J = 10.5, 5.8 Hz; 1H), 3.05 (td, ^3J = 10.9, 4.4 Hz; 1H), 2.43 (td, ^3J = 12.6, 3.3 Hz; 1H), 2.12–2.02 (m, 4H, including s: 2.05, Ac), 1.84 (t, ^3J = 11.5 Hz; 1H), 1.80–1.20 (m, 20H, including s: 1.73, CH_3), 1.14–0.93 (m, 6H, including s: 1.00, CH_3), 0.87–0.73 (m, 13H, including 4 s: 0.83, 0.82, 0.82, 0.78, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.52, 169.20, 160.18, 158.53, 150.39, 110.98, 110.09, 81.29, 55.58, 50.62, 50.40, 49.85, 46.92, 42.92, 40.98, 39.89, 38.54, 37.95, 37.75, 37.27, 34.51, 34.41, 30.25, 28.63, 28.09, 25.51, 23.83, 21.48, 21.02, 19.66, 18.30, 16.62, 16.31, 16.14, 15.04; IR (neat): 3070, 2950, 2870, 2585, 1730, 1675, 1640, 1470, 1375, 1290, 1230, 1200, 1030, 980, 880, 735 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{51}NO_5+Na]^+$ 588.3659; found 588.3636.

3-{(17S)-[3β-Acetyloxy-28-norlupane]-17-yl}-(isoxazol-5-yl) carboxylic acid 26: A solution of $NaClO_2$ (0.835 g, 9.230 mmol, 8 equiv. (79% purity by iodometric titration)) and NaH_2PO_4 (0.960 g, 8.080 mmol, 7 equiv.) in 20 mL of water was added dropwise to a stirred mixture of aldehyde **24** (0.625 g, 1.150 mmol, 1 equiv.) and 2,3-dimethyl-2-butene (8 mL) in THF/**t**-BuOH (1:1, 44 mL). After stirring at room temperature for 2 h, EtOAc (250 mL) was added to reaction mixture, reaction mixture was washed with water (2× 30 mL) and brine (3×40 mL), aqueous layer was extracted with CH_2Cl_2 (5×25 mL); combined organic fractions were dried over Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by RP C18 silica column chromatography (50:50→80:20 MeOH/H_2O + 0.1% AcOH) giving 229 mg (35%) of **26** as a colorless amorphous compound. R_f = 0.34 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.92 (s, 1H), 4.47 (dd, ^3J = 10.7, 5.5 Hz, 1H), 2.42–2.32 (m, 1H), 2.31–2.22 (m, 1H), 2.18–2.10 (m, 1H), 2.05 (s, 3H, Ac-, 1.94–1.82 (m, 1H), 1.77–1.17 (m, 19H), 1.11–1.04 (m, 1H), 1.03–0.67 (m, 23H; including 4 s: 0.98, 0.83, 0.82, 0.73, CH_3 and 0.87 (d, ^3J = 6.7 Hz; CH_3)); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.51, 169.36, 160.15, 158.54, 110.98, 81.27, 55.54, 50.71, 50.34, 49.35, 44.26, 43.12, 40.96, 40.10, 38.52, 37.94, 37.61, 37.23, 34.45, 34.34, 30.01, 28.63, 28.08, 26.91, 23.82, 23.15, 22.39, 21.47, 21.03, 18.30, 16.63, 16.25, 16.14, 15.02, 14.99; IR (neat): 2945, 2865, 1730, 1680, 1465, 1445, 1385, 1290, 1035, 980, 910, 730 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{35}H_{53}NO_5+H]^+$ 568.3997; found 568.4019.

3-{(17S)-[3β-Hydroxy-28-norlupan-20(29)-en]-17-yl}-(isoxazol-5-yl) carboxylic acid 17: KOH (0.112 g, 2.346 mmol, 8 equiv.) is added to solution of **25** (0.166 g, 0.293 mmol, 1 equiv.) in EtOH (8 mL). Reaction was stirred at 50 °C for 15 h, the reaction was quenched by addition of AcOH till pH 6 and evaporated in vacuum. The residue was purified by RP C18 silica column chromatography (60:40→95:5 MeOH/H_2O + 0.1% AcOH) giving 152 mg (99%) of betulin-acid **17** as a colorless amorphous compound.

A solution of $NaClO_2$ (0.223 g, 1.969 mmol, 8 equiv. (79% purity by iodometric titration)) and NaH_2PO_4 (0.258 g, 1.722 mmol, 7 equiv.) in 3 mL of water was added dropwise to a stirred mixture of aldehyde **15** (0.125 g, 0.246 mmol, 1 equiv.) and 2,3-dimethyl-2-butene (1.5 mL) in THF/**t**-BuOH (1:1, 8 mL). After stirring at room temperature for 3 h, AcOH (0.1 mL) was added to reaction mixture, reaction was evaporated in vacuum. The residue was purified by RP C18 silica column chromatography (50:50→95:5 MeOH/H_2O + 0.1% AcOH) giving 102 mg (79%) of **17** as a colorless amorphous compound.

^{1}H NMR (500 MHz, [D_6]DMSO): δ = 6.58 (s, 1H), 4.71 (s, 1H), 4.57 (s, 1H), 3.03–2.89 (m, 2H), 2.38 (td, ^3J = 12.1, 3.1 Hz; 1H), 2.05 (dt, ^2J = 13.2 Hz, ^3J = 3.0 Hz; 1H), 1.73 (t, ^3J = 11.4 Hz; 1H), 1.70–1.06 (m, 20H, including s: 1.67, CH_3), 1.06–0.89 (m, 5H, including s: 0.95, CH_3), 0.88–0.77 (m, 4H, including s: 0.84, CH_3), 0.72 (s, 3H,CH_3), 0.69 (s, 3H,

CH_3), 0.66–0.57 (m, 4H, including s: 0.62, CH_3); ^{13}C NMR (126 MHz, [D_6]DMSO): δ = 171.97, 167.81, 158.63, 150.28, 109.69, 105.96, 76.77, 54.88, 49.90, 49.44, 49.13, 46.48, 42.26, 40.28, 38.47, 38.22, 36.95, 36.70, 33.87, 33.54, 29.72, 28.08, 28.02, 27.14, 24.94, 21.06, 20.42, 19.11, 17.93, 15.87, 15.76, 15.71, 14.61; IR (neat): 3435, 3360, 2940, 2865, 1700, 1610, 1575, 1450, 1365, 1285, 1255, 1050, 980, 880, 795 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{49}NO_4+H]^+$ 524.3734; found 524.3727.

3-{(17S)-[3β-Hydroxy-28-norlupane]-17-yl}-(isoxazol-5-yl) carboxylic acid 18: KOH (0.150 g, 2.673 mmol, 11 equiv.) is added to solution of **26** (0.138 g, 0.243 mmol, 1 equiv.) in MeOH (15 mL). Reaction was stirred at 50 °C for 3 h, the reaction was quenched by addition of AcOH till pH 6 and evaporated in vacuum. The residue was purified by RP C18 silica column chromatography (60:40→90:10 MeOH/H_2O + 0.1% AcOH) giving 97 mg (76%) of betulin-acid **18** as a colorless amorphous compound.

A solution of $NaClO_2$ (0.177 g, 1.569 mmol, 8 equiv. (79% purity by iodometric titration)) and NaH_2PO_4 (0.206 g, 1.372 mmol, 7 equiv.) in 3 mL of water was added dropwise to a stirred mixture of aldehyde **16** (0.100 g, 0.196 mmol, 1 equiv.) and 2,3-dimethyl-2-butene (1.5 mL) in THF/**t**-BuOH (1:1, 8 mL). After stirring at room temperature for 3 h, AcOH (0.1 mL) was added to reaction mixture, reaction was evaporated in vacuum. The residue was purified by RP C18 silica column chromatography (60:40→90:10 MeOH/H_2O + 0.1% AcOH) giving 98 mg (95%) of **18** as a colorless amorphous compound.

R_f = 0.43 (CH_2Cl_2/MeOH 6:1); ^1H NMR (500 MHz, [D_6]DMSO): δ = 6.73 (s, 1H), 2.99–2.91 (m, 1H), 2.38–2.29 (m, 1H), 2.22–2.15 (m, 1H), 2.15–2.07 (m, 1H), 1.84–1.74 (m, 1H), 1.65–1.02 (m, 19H), 1.01–0.56 (m, 24H; including 5 s: 0.93, 0.83, 0.75, 0.65, 0.62, CH_3 and 0.77 (d, ^3J = 6.4 Hz; CH_3)); ^{13}C NMR (126 MHz, [D_6]DMSO): δ = 168.16, 164.65, 158.93, 106.62, 76.87, 54.99, 49.89, 49.79, 48.82, 43.78, 42.57, 40.41, 39.76, 38.58, 38.40, 36.90, 36.80, 34.19, 33.59, 29.61, 28.21, 28.17, 27.15, 26.55, 23.11, 22.10, 20.60, 18.06, 15.94, 15.92, 15.83, 14.87, 14.69; IR (neat): 3430, 2945, 2870, 1690, 1620, 1610, 1585, 1465, 1365, 1290, 1255, 1030, 985, 795, 780, 750 cm^{-1}; HRMS (ESI): *m/z* calcd. for $[C_{33}H_{51}NO_4+H]^+$ 526.3891; found 526.3914.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] a) E. W. H. Hayek, U. Jordis, W. Moche, F. Sauter, *Phytochemistry* **1989**, *28*, 2229–2242; b) S. Jager, M. N. Laszczyk, A. Scheffler, *Molecules* **2008**, *13*, 3224–3235.

[2] a) A. Yu. Spivak, R. R. Khalitova, D. A. Nedopekina, R. R. Gubaidullin, *Steroids* **2020**, *154*, 108530; b) S. Haque, D. A. Nawrot, S. Alakurtti, L. Ghemtio, J. Yli-Kauhaluoma, P. Tammela, *PLoS One* **2014**, *9*, e102696; c) O. Kazakova, T. Lopatina, G. Giniyatullina, M. Mioc, C. Soica, *Bioorg. Chem.* **2020**, *104*, 104209.

[3] a) A. Karagöz, M. Leidenberger, F. Hahn, F. Hampel, O. Friedrich, M. Marschall, B. Kappes, S. B. Tsogoeva, *Bioorg. Med. Chem.* **2019**, *27*, 110–115; b) J. T. Banzouzi, P. Njomnang Soh, S. Ramos, P. Toto, A. Cavé, J. Hemez, F. Benoit-Vical, *J. Ethnopharmacol.* **2015**, *173*, 100–104; c) G. N. Silva, D. C. Schuck, L. N. Cruz, M. S. Moraes, M. Nakabashi, G. Gosmann, C. R. Garcia, S. C. Gnoatto, *Trop. Med. Int. Health* **2015**, *20*, 29–39.

[4] a) X. Ci, J. Zhou, H. Lv, Q. Yu, L. Peng, S. Hua, *Cell Death Dis.* **2017**, *8*, *e2798*; b) M. C. Lingaraju, N. N. Pathak, J. Begum, V. Balaganur, H. D. Ramachandra, R. A. Bhat, M. Ram, V. Singh, K. Kandasamy, D. Kumar, *Eur. J. Pharm. Sci.* **2015**, *70*, 12–21; c) M. A. Nader, H. N. Baraka, *Eur. J. Pharm. Sci.* **2012**, *46*, 106–113; d) T. S. Khlebnicova, Y. A. Piven, F. A. Lakhvich, I. V. Sorokina, T. S. Frolova, D. S. Baev, T. G. Tolstikova, *Anti-Inflammatory Anti-Allergy Agents Med. Chem.* **2020**, *19*, 254–267.

[5] a) F. S. G. Silva, P. J. Oliveira, M. F. Duarte, *J. Agric. Food Chem.* **2016**, *64*, 2991–3008; b) R. Vinayagam, J. Xiao, B. Xu, *Phytochem. Rev.* **2017**, *16*, 535–553; c) A. J. G. Castro, L. H. Cazarolli, L. C. Bretanha, P. M. Sulis, D. P. R. Padilla, D. M. A. Novoa, B. F. Dambros, M. G. Pizzolatti, F. R. M. B. Silva, *Arch. Biochem. Biophys.* **2018**, *648*, 20–26; d) C. Genet, A. Strehle, C. Schmidt, G. Boudjelal, A. Lobstein, K. Schoonjans, M. Souchet, J. Auwerx, R. Saladin, A. Wagner, *J. Med. Chem.* **2010**, *53*, 178–190.

[6] a) C. Thomas, A. Gioiello, L. Noriega, A. Strehle, J. Oury, G. Rizzo, A. Macchiarulo, H. Yamamoto, C. Mataki, M. Pruzanski, R. Pellicciari, J. Auwerx, K. Schoonjans, *Cell Metab.* **2009**, *10*, 167–177; b) H. Y. Quan, D. Y. Kim, S. J. Kim, H. K. Jo, G. W. Kim, S. H. Chung, *Biochem. Pharmacol.* **2013**, *85*, 1330–1340.

[7] a) D. E. Martin, K. Salzwedel, G. P. Allaway, *Antivir. Chem. Chemoth.* **2008**, *19*, 107–113; b) D. Wang, W. Lu, F. Li, *Acta Pharm. Sin. B* **2015**, 5, 493–499.

[8] E. R. Trumbull, E. Bianchi, D. J. Eckert, R. M. Wiedhopf, J. R. Cole, *J. Pharm. Sci.* **1976**, *65*, 1407–1408.

[9] S. Amiri, S. Dastghaib, M. Ahmadi, P. Mehrbod, F. Khadem, H. Behrouj, M. R. Aghanoori, F. Machaj, M. Ghamsari, J. Rosik, A. Hudecki, A. Afkhami, M. Hashemi, M. J. Los, P. Mokarram, T. Madrakian, S. Ghavami, *Biotechnol. Adv.* **2020**, *38*, 107409.

[10] a) M. Kvasnica, M. Urban, N. J. Dickinson, J. Sarek, *Nat. Prod. Rep.* **2015**, ***32***, 1303–1330; b) H. W. Cui, Y. He, J. Wang, W. Gao, T. Liu, M. Qin, X. Wang, C. Gao, Y. Wang, M. Y. Liu, Z. Yi, W. W. Qiu, *Eur. J. Med. Chem.* **2015**, *95*, 240–248; c) M. Urban, M. Vlk, P. Dzubak, M. Hajduch, J. Sarek, *Bioorg. Med. Chem.* **2012**, *20*, 3666–3674; d) H. D. Thi, N. T. K. Tuyet, C. P. The, H. T. Nguyen, C. B. Thi, T. D. Duy, M. D'hooghe, T. V. Nguyen, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5190–5194.

[11] a) S. Xiao, Q. Wang, L. Si, Y. Shi, H. Wang, F. Yu, Y. Zhang, Y. Li, Y. Zheng, C. Zhang, C. Wang, L. Zhang, D. Zhou, *ChemMedChem* **2014**, *9*, 1060–1070; b) P. Suman, A. Patel, L. Solano, G. Jampana, Z. S. Gardner, C. M. Holt, S. C. Jonnalagadda, *Tetrahedron* **2017**, *73*, 4214–4226; c) B. Pattnaik, J. K. Lakshmi, R. Kavitha, B. Jagadeesh, D. Bhattacharjee, N. Jain, U. V. Mallavadhani, *J. Asian Nat. Prod. Res.* **2017**, *19*, 260–271; d) R. Majeed, A. Hussain, P. L. Sangwan, P. K. Chinthakindi, I. Khan, P. R. Sharma, S. Koul, A. K. Saxena, A. Hamid, *Mol. Carcinog.* **2016**, *55*, 964–976; e) D. Bori, H.-Y. Hung, K. Qian, C.-H. Chen, S. L. MorrisNatschke, K.-H. Lee, *Tetrahedron Lett.* **2012**, *53*, 1987–1989; f) R. Majeed, P. L. Sangwan, P. K. Chinthakindi, I. Khan, N. A. Dangroo, N. Thota, A. Hamid, P. R. Sharma, A. K. Saxena, S. Koul, *Eur. J. Med. Chem.* **2013**, *63*, 782–792; g) I. Govdi, N. V. Sokolova, I. V. Sorokina, D. S. Baev, T. G. Tolstikova, V. I. Mamatyuk, D. S. Fadeev, S. F. Vasilevsky, V. G. Nenadjenko, *MedChemComm* **2015**, *6*, 230–238; h) E. F. Khusnutdinova, P. Bremond, A. V. Petrova, O. S. Kukovinets, O. B. Kazakova, *Lett. Org. Chem.* **2017**, *14*, 743–747.

[12] a) J. Akhtar, A. A. Khan, Z. Ali, R. Haider, M. S. Yar, *Eur. J. Med. Chem.* **2017**, *125*, 143–189; b) M. Wan, L. Xu, L. Hua, A. Li, S. Li, W. Lu, Y. Pang, C. Cao, X. Liu, P. Jiao, *Bioorg. Chem.* **2014**, *54*, 38–43; c) R. Shakeel, R. Masood, V. K. Tripathi, J. Singh, T. Ara, S. Koul, S. Farooq, A. Kaul, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4243–4246; d) K. R. Reddy, P. S. Rao, G. J. Dev, Y. Poornachandra, C. G. Kumar, P. S. Rao, B. Narsaiah, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1661–1663; e) S. Tapadar, R. He, D. N. Luchini, D. D. Billadeau, A. P. Kozikowski, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3023–3026; f) J. Mao, H. Yuan, Y. Wang, B. Wan, M. Pieroni, Q. Huang, R. B.

Breemen, A. P. Kozikowski, S. G. Franzblau, *J. Med. Chem.* **2009**, *52*, 6966–6978; g) A. Kamal, E. V. Bharathi, J. S. Reddy, M. J. Ramaiah, D. Dastagiri, M. K. Reddy, A. Viswanath, T. L. Reddy, T. B. Shaik, S. Pushpavalli, M. P. Bhadra, *Eur. J. Med. Chem.* **2011**, *46*, 691–703; h) P.-A. Fournier, M. Arbour, E. Cauchon, A. Chen, A. Chefson, Y. Ducharme, J.-P. Falgueyret, S. Gagné, E. Grimm, Y. Han, R. Houle, P. Lacombe, J.-F. Lévesque, D. MacDonald, B. Mackay, D. McKay, M. D. Percival, Y. Ramtohul, R. St-Jacques, S. Toulmond, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2670–2674; i) C. Zhang, X. Wang, H. Liu, M. Zhang, M. Geng, L. Sun, A. Shen, A. Zhang, *Eur. J. Med. Chem.* **2017**, *125*, 315–326.

[13] a) S. Sun, Q. Jia, Z. Zhang, *Bioorg. Med. Chem. Lett.* **2019**, *29*, 2535–2550; b) T.-F. Niu, M.-F. Lv, L. Wang, W.-B. Yi, C. Cai, *Org. Biomol. Chem.* **2013**, *11*, 1040–1048.

[14] V. V. Grishko, I. A. Tolmacheva, V. O. Nebogatikov, N. V. Galaiko, A. V. Nazarov, M. V. Dmitriev, I. B. Ivshina, *Eur. J. Med. Chem.* **2017**, *125*, 629–639.

[15] a) Y.-J. You, Y. Kim, N.-H. Nam, B.-Z. Ahn, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3137–3140; b) A. Koohang, N. D. Majewski, E. L. Szotek, A. A. Mar, D. A. Eiznhamer, M. T. Flavin, Z.-Q. Xu, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2168–2171; c) A. Minassi, F. Rogati, C. Cruz, M. E. Prados, N. Galera, C. Jinénez, G. Appendino, M. L. Bellido, M. A. Calzado, D. Caprioglio, E. Muñoz, *J. Nat. Prod.* **2018**, *81*, 2235–2243; d) J. Xu, Z. Li, J. Luo, F. Yang, T. Liu, M. Liu, W.-W. Qiu, J. Tang, *J. Med. Chem.* **2012**, *55*, 3122–3134.

[16] A. N. Antimonova, N. I. Petrenko, M. M. Shakirov, M. A. Pokrovskii, A. G. Pokrovskii, E. E. Shul'ts, *Chem. Nat. Compd.* **2014**, *50*, 1016–1023.

[17] a) F. Hu, M. Szostak, *Adv. Synth. Catal.* **2015**, *357*, 2583–2614; b) J. Plumet, *ChemPlusChem* **2020**, *85*, 2252–2271.

[18] a) J. Lugiņina, V. Rjabovs, S. Belyakov, M. Turks, *Tetrahedron Lett.* **2013**, *54*, 5328–5331; b) J. Kurkowska, I. Zadrozna, *J. Chem. Research (S)* **2003**, 254–255; c) S. Chimichi, M. Boccalini, B. Cosimelli, F. Dall'Acquac, G. Violac, *Tetrahedron* **2003**, *59*, 5215–5223.

[19] a) P. Kumar, M. Kapur, *Org. Lett.* **2019**, *21*, 2134–2138; b) T. Radhikaa, A. Vijaya, B. V. Harinadhaa, B. Madhavareddya, *Russ. J. Bioorg. Chem.* **2020**, *46*, 429–437; c) F. Xie, T. Ni, Z. Ding, Y. Hao, R. Wang, R. Wang, T. Wang, X. Chai, S. Yu, Y. Jin, Y. Jiang, D. Zhang, *Bioorg. Chem.* **2020**, *101*, 103982.

[20] a) T. von Zons, L. Brokmann, J. Lippke, T. Preusse, M. Huelsmann, A. Schaate, P. Behrens, A. Godt, *Inorg. Chem.* **2018**, *57*, 3348–3359; b) S. Bhosale, S. Kurhade, U. V. Prasad, V. P. Palle, D. Bhuniya, *Tetrahedron Lett.* **2009**, *50*, 3948–3951; c) O. V. Demina, A. A. Khodonov, E. I. Sinauridze, V. I. Shvets, S. D. Varfolomeeva, *Russ. Chem. Bull.* **2014**, *63*, 2092–2113; d) M. Flipo, M. Desroses, N. Lecat-Guillet, B. Villemagne, N. Blondiaux, F. Leroux, C. Piveteau, V. Mathys, M.-P. Flament, J. Siepmann et al., *J. Med. Chem.* **2012**, *55*, 68–83; e) L. Han, B. Zhang, M. Zhu, J. Yan, *Tetrahedron Lett.* **2014**, *55*, 2308–2311; f) A. M. Jawalekar, E. Reubsaet, F. P. J. T. Rutjes, F. L. van Delft, *Chem. Commun.* **2011**, *47*, 3198–3200; g) M. Kim, Y. S. Hwang, W. Cho, S. B. Park, *ACS Comb. Sci.* **2017**, *19*, 407–413; h) O. Moriya, H. Takenaka, M. Iyoda, Y. Urata, T. Endo, *J. Chem. Soc. Perkin Trans. 1* **1994**, 413–417; i) A. Oancea, E. Georgescu, F. Georgescu, A. Nicolescu, E. I. Oprita, C. Tudora, L. Vladulescu, M.-C. Vladulescu, F. Oancea, C. Deleanu, *Beilstein J. Org. Chem.* **2017**, *13*, 659–664.

[21] A. Wiebe, T. Gieshoff, S. Möhle, E. Rodrigo, M. Zirbes, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 5594–5619; *Angew. Chem.* **2018**, *130*, 5694–5721.

[22] S. Möhle, M. Zirbes, E. Rodrigo, T. Gieshoff, A. Wiebe, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 6018–6041; *Angew. Chem.* **2018**, *130*, 6124–6149.

[23] a) M. Yan, Y. Kawamata, P. S. Baran, *Chem. Rev.* **2017**, *117*, 13230–13319; b) H. Kolbe, *J. Prakt. Chem.* **1847**, *41*, 137–139.

[24] B. A. Frontana-Uribe, R. D. Little, J. G. Ibanez, A. Palma, R. Vasquez-Medrano, *Green Chem.* **2010**, *12*, 2099–2119.

[25] A. Kirste, G. Schnakenburg, F. Stecker, A. Fischer, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 971–975; *Angew. Chem.* **2010**, *112*, 983–987.

[26] a) H.-J. Schäfer in *Topics in Current Chemistry* (Eds.: M. J. S. Dewar, J. D. Dunitz, K. Hafner, E. Heilbronner, Ś. Itô, J.-M. Lehn, K. Niedenzu, K. N. Raymond, C. W. Rees, F. Vögtle et al.), Springer Berlin Heidelberg, Berlin, Heidelberg, **1987**, pp. 101–129; b) S. B. Beil, M. Breiner, L. Schulz, A. Schüll, T. Müller, D. Schollmeyer, A. Bomm, M. Holtkamp, U. Karst, W. Schade, S. R. Waldvogel, *RSC Adv.* **2020**, *10*, 14249–14253; c) S. B. Beil, T. Müller, S. B. Sillart, P. Franzmann, A. Bomm, M. Holtkamp, U. Karst, W. Schade, S. R. Waldvogel, *Angew. Chem. Int. Ed.* **2018**, *57*, 2450–2454; *Angew. Chem.* **2018**, *130*, 2475–2479.

[27] R. Francke, T. Quell, A. Wiebe, S. R. Waldvogel in *Organic electrochemistry* (Eds.: O. Hammerich, B. Speiser), CRC Press, Boca Raton, London, New York, **2016**, pp. 981–1033.

[28] L. J. Wesenberg, E. Diehl, T. J. B. Zähringer, C. Dörr, D. Schollmeyer, A. Shimizu, J. Yoshida, U. A. Hellmich, S. R. Waldvogel, *Chem. Eur. J.* **2020**, *26*, 17574–17580.

[29] Renewables 2019 Global Status Report; REN21 Secretariat: Paris, France, 2019; Available online: http://www.ren21.net/gsr-2019/ (accessed 04 March 2021).

[30] a) D. Pollok, S. R. Waldvogel, *Chem. Sci.* **2020**, *11*, 12375–12592; b) J. Seidler, J. Strugatchi, T. Gärtner, S. R. Waldvogel, *MRS Energy Sustainability#j.hofmann - 16.08.2017 11:12:13* **2020**, *7*, E42.

[31] N. Melnikova, I. Burlova, T. Kiseleva, I. Klabukova, M. Gulenova, C. A. C. Kislitsin, V. Vasin, B. Tanaseichuk, *Molecules* **2012**, *17*, 11849–11863.

[32] A. Ortiz, M. Soumeillant, S. A. Savage, N. A. Strotman, M. Haley, T. Benkovics, J. Nye, Z. Xu, Y. Tan, S. Ayers et al., *J. Org. Chem.* **2017**, *82*, 4958–4963.

[33] R. Csuk, K. Schmuck, R. Schäfer, *Tetrahedron Lett.* **2006**, *47*, 8769–8770.

[34] A. Barthel, S. Stark, R. Csuk, *Tetrahedron* **2008**, *64*, 9225–9229.

[35] M. M. Iftime, L. Marin, *Ultrason. Sonochem.* **2018**, *45*, 238–247.

[36] R. Doi, M. Shibuya, T. Murayama, Y. Yamamoto, Y. Iwabuchi, *J. Org. Chem.* **2015**, *80*, 401–413.

[37] H. L. Ziegler, H. Franzyk, M. Sairafianpour, M. Tabatabai, M. D. Tehrani, K. Bagherzadeh, H. Hägerstrand, D. Staerk, J. W. Jaroszewski, *Bioorg. Med. Chem.* **2004**, *12*, 119–127.

[38] J. Yli-Kauhaluoma, S. Alakurtti, J. Minkkinen, N. Sarcerdoti-Sierra, C. L. Jaffe, T. Heiska, PTC/FI2007/050331.

[39] N. G. Komissarova, N. G. Belenkova, L. V. Spirikhin, O. V. Shitikova, M. S. Yunusov, *Chem. Nat. Compd.* **2002**, *38*, 58–61.

[40] R. Haavikko, A. Nasereddin, N. Sacerdoti-Sierra, D. Kopelyanskiy, S. Alakurtti, M. Tikka, C. L. Jaffe, J. Yli-Kauhaluoma, *MedChemComm* **2014**, *5*, 445–451.

[41] a) L. Pohjala, S. Alakurtti, T. Ahola, J. Yli-Kauhaluoma, P. Tammela, *J. Nat. Prod.* **2009**, *72*, 1917–1926; b) N. Antimonova, N. V. Uzenkova, N. I. Petrenko, M. M. Shakirov, E. E. Shul'ts, G. A. Tolstikov, *Russ. J. Org. Chem.* **2011**, *47*, 589–601.

[42] K. Hata, K. Hori, S. Takahashi, *J. Nat. Prod.* **2002**, *65*, 645–648.

[43] A. Pichette, H. Liu, C. Roy, S. Tanguay, F. Simard, S. Lavoie, *Synth. Commun.* **2004**, *34*, 3925–3937.

[44] a) R. Csuk, A. Barthel, S. Schwarz, H. Kommera, R. Paschke, *Bioorg. Med. Chem.* **2010**, *18*, 2549–2558; b) P. Mäki-Arvela, M. Barsukova, I. Winberg, A. Smeds, J. Hemming, K. Eränen, A. Torozova, A. Aho, K. Volcho, D. Y. Murzin, *ChemistrySelect* **2016**, *1*, 3866–3869.

[45] J. Holy, O. Kolomitsyna, D. Krasutsky, P. J. Oliveira, E. Perkins, P. A. Krasutsky, *Bioorg. Med. Chem.* **2010**, *18*, 6080–6088.

[46] P. A. Krasutsky, A. B. Khotkevych, A. Pushechnikov, A. Rudnitskaya, PCT/US2006/011794.

[47] H. Menard, C. M. Cirtiu, J.-M. Lalancette, L. Ruest, Z. Kaljaca, PCT/CA2005/001919.

[48] a) M. Ishihara, H. Sakagami, W.-K. Liu, *Anticancer Res.* **2005**, *25*, 3951–3955; b) J. Achrem-Achremowicz, E. Kępczyńska, M. Zylewski, Z. Janeczko, *Biomed. Chromatogr.* **2010**, *24*, 261–267.

[49] L. M. Reid, T. Li, Y. Cao, C. P. Berlinguette, *Sustain. Energy Fuels* **2018**, *2*, 1905–1927.

[50] R. Francke, R. D. Little, *Chem. Soc. Rev.* **2014**, *43*, 2492–2521.

[51] a) N. Yang, S. Yu, J. V. Macpherson, Y. Einaga, H. Zhao, G. Zhao, G. M. Swain, X. Jiang, *Chem. Soc. Rev.* **2019**, *48*, 157–204; b) S. R. Waldvogel, S. Mentizi, A. Kirste, *Top. Curr. Chem.* **2012**, *320*, 1–32; c) S. Lips, S. R. Waldvogel, *ChemElectroChem* **2019**, *6*, 1649–1660.

[52] a) B. Elsler, D. Schollmeyer, K. M. Dyballa, R. Franke, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 7122–7123; Angew . *Chem.* **2014**, *126*, 7248–7249; b) S. Lips, D. Schollmeyer, R. Franke, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 13325–13329; *Angew. Chem.* **2018**, *130*, 13509–13513; c) S. Lips, A. Wiebe, B. Elsler, D. Schollmeyer, K. M. Dyballa, R. Franke, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 10872–10876; *Angew. Chem.* **2016**, *128*, 11031–11035; d) E. Rodrigo, H. Baunis, E. Suna, S. R. Waldvogel, *Chem. Commun.* **2019**, *55*, 12255–12258; e) E. Rodrigo, S. R. Waldvogel, *Green Chem.* **2018**, *20*, 2013–2017; f) A. Wiebe, S. Lips, D. Schollmeyer, R. Franke, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 14727–14731; *Angew. Chem.* **2017**, *129*, 14920–14925; g) A. Wiebe, B. Riehl, S. Lips, R. Franke, S. R. Waldvogel, *Sci. Adv.* **2017**, *3*, eaao3920; h) A. Wiebe, D. Schollmeyer, K. M. Dyballa, R. Franke, S. R. Waldvogel, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 11801–11805; *Angew. Chem.* **2016**, *128*, 11979–11983.

[53] a) J. E. Nutting, M. Rafiee, S. S. Stahl, *Chem. Rev.* **2018**, *118*, 4834–4885; b) C. Deckers, M. Linden, H. Löwe, *Chem. Eng. Technol.* **2019**, *42*, 2044–2051.

[54] J. E. Nutting, M. Rafiee, S. S. Stahl, *Chem. Rev.* **2018**, *118*, 4834–4885.

[55] A. Wahhab, M. Ottosen, F. W. Bachelor, *Can. J. Chem.* **1991**, *69*, 570–577.
[56] a) K.-C. Liu, B. R. Shelton, R. K. Howe, *J. Org. Chem.* **1980**, *45*, 3916–3918; b) H.-J. Gi, Y. Xiang, R. F. Schinazi, K. Zhao, *J. Org. Chem.* **1997**, *62*, 88–92.
[57] a) S. K. Armstrong, E. W. Collington, J. G. Knight, A. Naylorb, S. Warren, *J. Chem. Soc. Perkin Trans. 1* **1993**, *1*, 1433–1447; b) A. Hassner, K. M. Lokanatha Rai, *Synthesis* **1989**, 57–59; c) G. Zhao, L. Liang, C. H. E. Wen, R. Tong, *Org. Lett.* **2019**, *21*, 315–319; d) L. Han, B. Zhang, C. Xiang, J. Yan, *Synthesis* **2014**, *46*, 503–509.
[58] J. Kiegiel, M. Popławska, J. Joźwik, M. Kosior, J. Jurczak, *Tetrahedron Lett.* **1999**, *40*, 5605–5608.
[59] A. Ortiz, M. Soumeillant, S. A. Savage, N. A. Strotman, M. Haley, T. Benkovics, J. Nye, Z. Xu, Y. Tan, S. Ayers, Q. Gao, S. Kiau, *J. Org. Chem.* **2017**, *82*, 4958–4963.
[60] a) G. W. Buchanan, B. A. Dawson, *Can. J. Chem.* **1978**, *56*, 2200–2204; b) R. Marek, A. Lyčka, *Curr. Org. Chem.* **2002**, *6*, 35–66; c) P. R. Seidl, J. F. Dias, "NMR Spectra of Hydroxylamines, Oximes and Hydroxamic Acids" in *PATAI'S Chemistry of Functional Groups*, Part 1. (Eds.: Z. Rappoport, J. F. Liebman), John Wiley & Sons, Ltd., **2009**, pp. 85–116.
[61] Deposition Number 2053830 (for **12**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.
[62] M. F. Hartmer, S. R. Waldvogel, *Chem. Commun.* **2015**, *51*, 16346–16348.
[63] T. Shono, Y. Matsumura, K. Tsubata, T. Kamada, K. Kishi, *J. Org. Chem.* **1989**, *54*, 2249–2251.
[64] C. Gütz, A. Stenglein, S. R. Waldvogel, *Org. Process Res. Dev.* **2017**, *21*, 771–778.
[65] a) K. Beltrop, P. Meister, S. Klein, A. Heckmann, M. Grünebaum, H.-D. Wiemhöfer, M. Winter, T. Placke, *Electrochim. Acta* **2016**, *209*, 44–55; b) J. Gao, S. Tian, L. Qi, H. Wang, *Electrochim. Acta* **2015**, *176*, 22–27; c) J. Gao, M. Yoshio, L. Qi, H. Wang, *J. Power Sources* **2015**, *278*, 452–457.
[66] S. B. Beil, D. Pollok, S. R. Waldvogel, *Angew. Chem. Int. Ed.* **2021**, *in press*. [DOI: 10.1002/anie.202014544].
[67] Deposition Number 2053832 (for **25**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.
[68] N. S. Sirazhetdinova, V. A. Savelyev, T. S. Frolova, D. S. Baev, L. S. Klimenko, I. V. Chernikov, O. S. Oleshko, T. A. Sarojan, A. G. Pokrovskii, E. E. Shults, *Molecules* **2020**, *25*, 2547.
[69] R. C. Santos, J. A. R. Salvador, S. Marín, M. Cascante, *Bioorg. Med. Chem.* **2009**, *17*, 6241–6250.
[70] C. R. Dorr, S. Yemets, O. Kolomitsyna, P. Krasutsky, L. M. Mansky, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 542–545.

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