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Isosteviol – A new scaffold for the synthesis of carbonic anhydrase II inhibitors

Toni C. Denner, Niels V. Heise, René Csuk *

Organic Chemistry, Martin–Luther–University Halle-Wittenberg, Kurt–Mothes Str. 2, D-06120 Halle (Saale), Germany

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ABSTRACT

The diterpene isosteviol can easily be synthesized via hydrolysis from stevioside, a renewable resource adding particular utility to its potential for drug synthesis. Of late, there has been an increase in scientific studies focusing on inhibitors of the enzyme carbonic anhydrase II, CA II, since this enzyme is not used just in glaucoma treatment but also in mitigating the side effects of Alzheimer's disease antibody therapy. The presence of a sulfamate or a sulfonamide group is a key structural feature in many CA II inhibitors. Thus, isosteviol was transformed into sulfamates, either spacered or unspacered, to scrutinize their ability to perform as CA II inhibitors. Three particular derivatives were discovered to be effective inhibitors. As a competitive inhibitor with a K_i = 2.59 μM, a 16-*O*-acetyl-isosteviol compound holding a 5-amino-1,3,4-thiadiazol-2-yl-amino substituent attached to a succinoyl ester nearly entirely inhibited CA II.

1. Introduction

Interest in the development of inhibitors of carbonic anhydrases (CAs) has increased in recent years. This newly awakened interest can be explained by several different facts. On the one hand, CAs are involved in a variety of physiological processes, including respiration, transportation of carbon dioxide and bicarbonate, pH and carbon dioxide homeostasis, gluconeogenesis, adipogenesis, ureagenesis as well as calcification [1–10]. As a result, the use of CAs for therapeutic purposes has led to the need to develop isoenzyme-specific inhibitors in order to minimize the type and number of possible interactions and undesirable side effects. In addition to an increased interest in inhibitors for the isoenzymes CA IX and CA XII, which are of particular importance in the treatment of hypoxic tumors, inhibitors of CA II, in particular, have recently gained in importance [11–18]. This can be explained on the one hand by their successful use in the treatment of eye diseases (e.g., glaucoma) [19–25] and on the other hand by the observation that CA inhibitors (CAIs) can also be applied to reduce oedema, in particular macular and cerebral oedema [26–31]. An abnormal level of hCA II has also been associated with neuropathic pain or epilepsy. Especially for the treatment of cerebral oedema it seems significant that the active substances lecanemab, aducanumab and donanemab recently used to treat Alzheimer's disease (AD) might lead to brain oedema and swelling in a significant number of patients as side effects, which can also result in death if not appropriately treated [32–37]. As the number of people suffering from AD or who will suffer from it in the next few years is very large worldwide and still increasing [38] (55 million in 2020, expected in 2030 78 million and 139 million in 2050), and the number of people who will be treated with these novel drugs will also increase, the development of CA II inhibitors seems of the highest interest.

Over the years, many different structures have been considered. What many structures, however, have in common is that they hold a sulfamate group as the effective structural element. For example, methazolamide [39,40] is used to treat glaucoma and acute mountain disease, ethoxzolamide [41,42] found applications to treat duodenal ulcers, and indisulam [43–45] is applied in the therapy of melanoma and leucemia. In this context, acetazolamide (**AAZ**) [46–49], a relatively non-selective CAI, and **SLC-0111** [50–57] (Scheme 1), a particularly successful CAI in the treatment of cancer, are particularly worth mentioning. Previous studies have also shown that the backbone to which the sulfonamide or the sulfamate group is attached is of particular importance, too, especially concerning a cell/organ targeting of the compounds. Thereby, our own earlier studies were mainly focused on pentacyclic triterpenes, some of which proved to be particularly good inhibitors of CA IX and CA II [58–64]. However, a tacit prerequisite in the development of novel CAIs is also good = low-cost accessibility of the molecular target. This is often not the case with pentacyclic triterpenes, and individual representatives of this substance class (e.g.,

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

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betulinic acid, platanic acid) hold also poor solubility properties, which makes future therapeutic application much more difficult.

A relatively new basic structure is provided by the diterpene isosteviol, which can be obtained in very good yields by acid hydrolysis from the sweetener stevioside (Scheme 1); the latter is available in large quantities and at low cost. In contrast to many triterpenoidic scaffolds isosteviol is not cytotoxic. Stevioside is also to be regarded as a renewable raw material as it can usually be obtained by extraction from the easily cultivated plant *Stevia rebaudiana* Bertoni (Asteraceae) which is also known as sugar leaf.

2. Results and discussion

Stevioside (Scheme 1) was converted into isosteviol (**1**) by acid hydrolysis as previously reported. Extensive NMR spectroscopic investigations also allowed an unambiguous assignment of all signals in the ^{1}H and ^{13}C NMR spectra [65,66]. Thereby, the use of a 2D ^{13}C INADEQUATE experiment proved very helpful in the unambiguous assignment of all carbons especially of the methyl groups. The NMR data for **1** have been compiled in Table. 1.

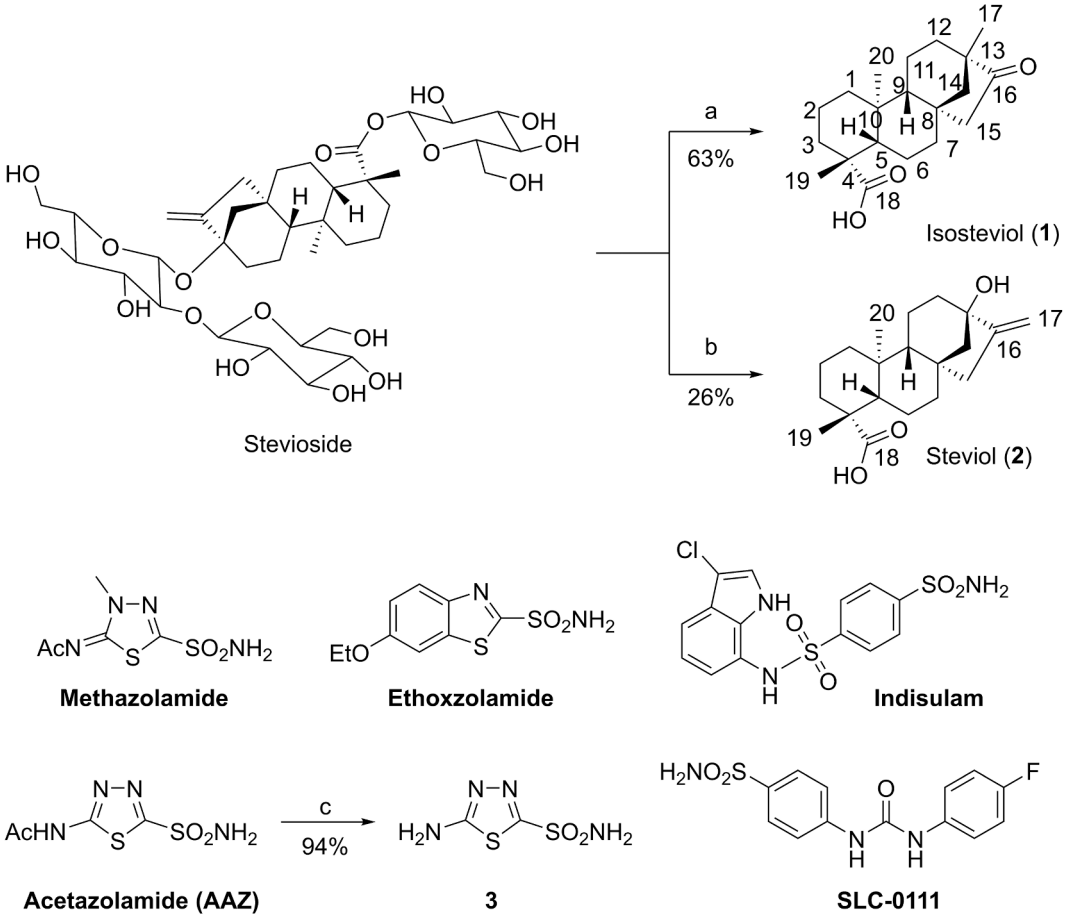
These data served in turn as the basis for the assignment of the signals in the corresponding derivatives of this investigation. The reagents required for subsequent reactions, sulfamoyl chloride was bought from local vendors and 5-amino-1,3,4-thiadiazole-2-sulfonamide (**3**), was obtained following a known synthesis (Scheme 1) from acetazolamide (**AAZ**).

Reduction of isosteviol (**1**) with sodium borohydride in dry methanol (Scheme 2) gave "dihydro-isosteviol" (**4**) [67–69] in very good yields.

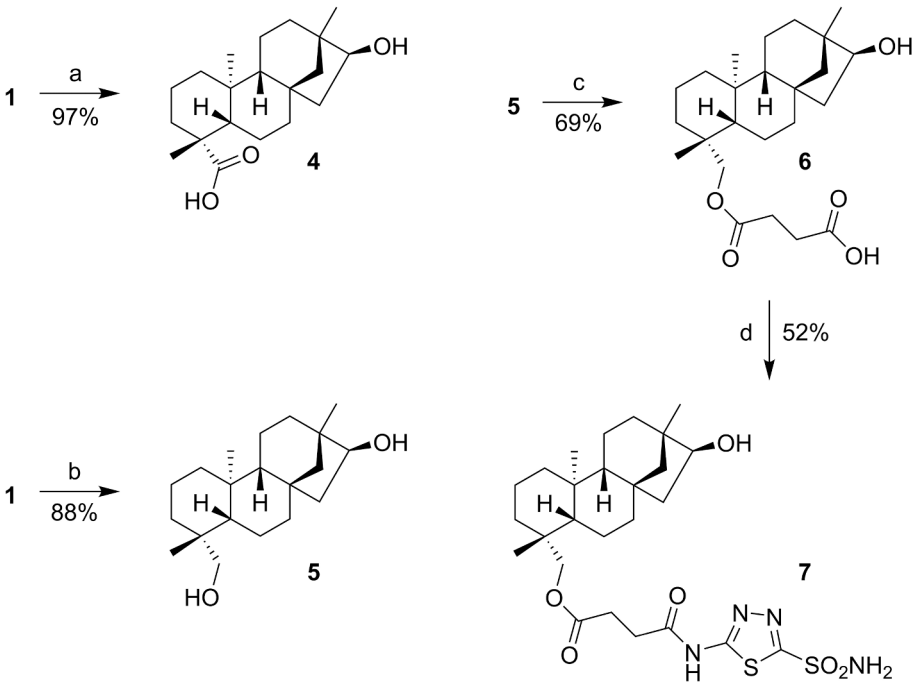
Table 1
Complete assignments of ^{1}H (500 MHz) and ^{13}C NMR (126 MHz) spectra for isosteviol (1) in $CDCl_3$; 21 °C; numbering as depicted in Scheme 1.

Atom	δ (^{13}C NMR) (ppm)	δ (^{1}H NMR) (ppm)	$^{3}J_{H,H}$ (Hz)
1	39.6	1.71; 0.90	13.3, 4.3
2	18.7	1.81; 1.41	
3	37.5	2.15, 1.01	13.1, 4.0; 13.6, 4.2
4	43.5	–	
5	56.9	1.14	12.1, 2.3
6	21.4	1.86; 1.73	
7	41.3	1.64; 1.48	13.3, 3.1; 13.6, 4.0
8	39.6	–	
9	54.6	1.16	
10	38.0	–	
11	20.2	1.67; 1.19	
12	37.2	1.59, 1.35	
13	48.6	–	
14	54.1	1.53; 1.40	11.6, 2.7; 11.4, 3.7
15	48.3	2.62; 1.80	18.6, 3.7; 18.5
16	222.7	–	
17	19.7	0.96	
18	183.9	–	
19	28.8	1.23	
20	13.2	0.77	

This reduction is highly selective and only traces of the corresponding diastereomeric alcohol could be detected by HPTLC-ESI-MS. The high selectivity of this reaction is in good agreement with previous reports, and can be explained by the fact that the borohydride anion attacks from the least sterically hindered side thus leading to a product holding a



Scheme 1. Synthesis of isosteviol (1) and steviol (2) from stevioside: a) MeOH, conc. HCl, reflux 2 h, 20 °C overnight, 63 %; b) H_2O; $NaIO_4$, KOH, 24 h, 21 °C, 26 %; c) synthesis of 3 from acetalzolamide (AAZ): conc. aq. HCl, reflux, 3 h, 94 %; structure of clinically used CA inhibitors methazolamide, ethoxzolamide, indisulam and SCC-0111.



Scheme 2. Reactions and conditions: a) $NaBH_4$, EtOH, 0 °C, 1 h, 97 %; b) $LiAlH_4$, THF, reflux, 3 h, 88 %; c) pyridine, DMAP (cat.), succinic anhydride, microwaves, 200 °C, 3 h, 69 %; d) THF, NMM, ethyl chloroformate, 20 °C, 15 min, then 3, reflux, 48 h, 52 %.

gauche orientation of the hydroxyl group attached to C-16 and the methyl group C-17. The structure of this product has also previously been confirmed by x-ray diffraction [67,68].

Reduction of **1** with lithium aluminum hydride gave diol **5** whose microwave assisted reaction with succinic anhydride in pyridine in the presence of cat. amounts of DMAP yielded 69 % of **6**. The position of the succinoyl residue was determined from 2D-NMR spectra; the preferential acylation at the C-18 position can be explained by the higher reactivity of the primary hydroxyl group compared to the secondary hydroxyl group at C-18. Reaction of **6** with ethyl chloroformate, 4-methyl-morpholine in dry THF followed by further reaction with **3** for 48 h under reflux led to the corresponding product **7**. The ^{1}H and ^{13}C NMR spectra of **7** show the typical signals for the diterpenoid backbone, the signals for the spacer (^{1}H NMR: CH_2: δ = 2.77–2.84 and δ = 2.71–2.64 ppm), and the thiadiazol moiety (^{13}C NMR: δ = 164.3 and 161.0 ppm). In the ESI-MS a m/z = 429.4 corresponds to a quasi-molecule ion $[M + Na]^+$, thereby additionally confirming the structure as do the results from the micro-analysis.

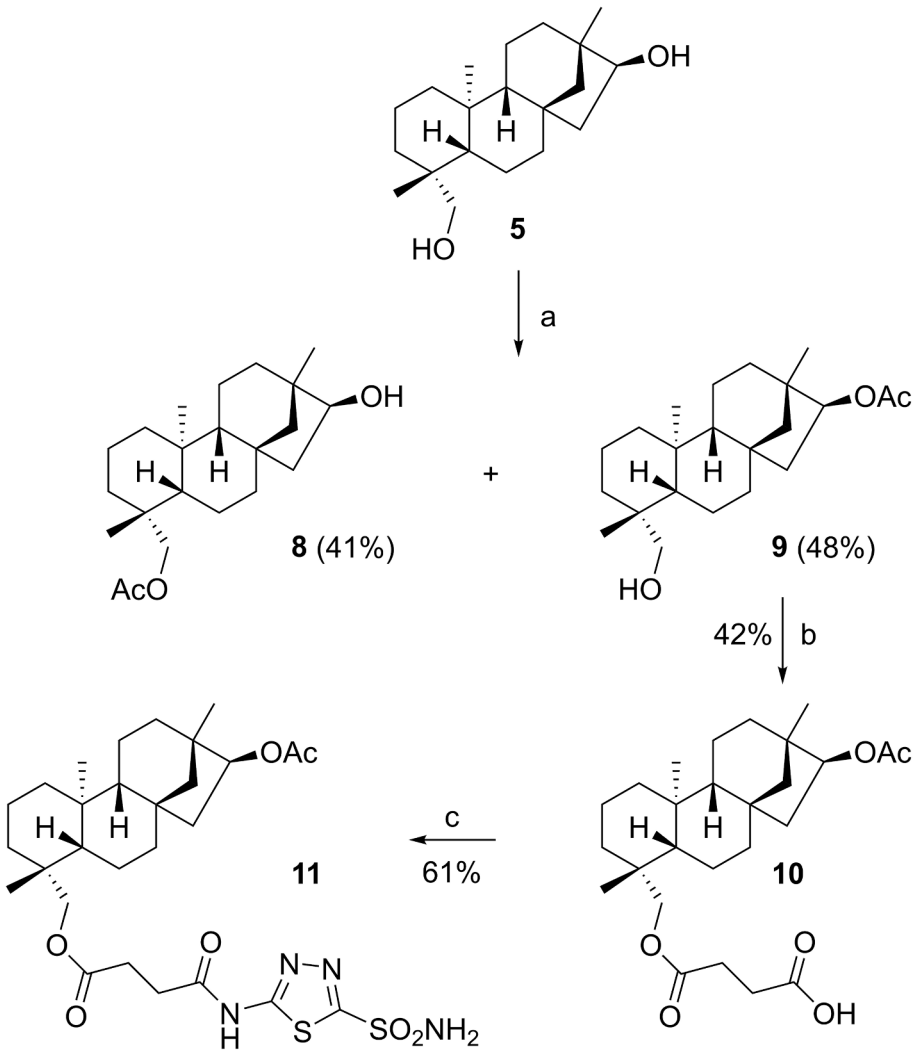
Acetylation of **5** (Scheme 3) gave a mixture of acetates **8** and **9** that were separated by chromatography. Similarly to the synthesis of **6**, compound **9** was converted into succinoyl-spacered **10** whose reaction with **3** afforded thiadiazol derivative **11** in 61 % isolated yield.

To determine the influence of the spacer and of the thiadiazole fragment, sulfamates were also prepared for comparison purposes by direct reaction (Scheme 4) of the alcohol with sulfamoyl chloride. Reaction of the mono-acetates **8** and **9** with sulfamoyl chloride gave the compounds **12** and **13** being acetylated/sulfamoylated at different positions, respectively. The target compound **15** (Scheme 4) was obtained from **4** under the same conditions. A double sulfamoylation reaction was carried out on **5**, and **14** was obtained in 83 % yield. For comparison, steviol (**2**) was sulfamoylated, and sulfamate **16** was obtained.

The compounds were screened for their ability to inhibit CA II. Thereby, acetazolamide (AAZ) – a sulfonamide type inhibitor with high affinity for CA II under physiological conditions, was used as a positive standard. The results are summarized in Table 2 and Fig. 1, which show that **11**, **12** and **15** are the best inhibitors of this enzyme, while **14** and **16** showed little activity. Compounds **12** and **13** differ only in the position of their acetate and sulfamate groups, but **12** is a better inhibitor than **13**; a sulfamate at position C-16 seems to be superior to a localization at C-18, and **15** also retained a good inhibitory activity. However, the presence of a second sulfamate group (as in **14**) led to an almost complete loss of activity. The same was true for the comparison between steviol-derived **16** (with almost no activity) and isosteviol-derived **15** (94.6 % inhibition of CA II). This difference underlines the significant impact of structural variations between the two scaffolds.

The moderate inhibitory activity of **7** suggests that the presence of an unprotected hydroxyl group may reflect greater flexibility or less favorable interactions with the active site of the enzyme, resulting in only moderate inhibition of the enzyme. The remarkable increase in inhibitory activity observed for **11** highlights the potent effect of protecting the hydroxyl group as an acetate. This modification is likely to enhance binding interactions, possibly through hydrogen bonding or hydrophobic interactions, resulting in almost complete inhibition. Compounds **11** and **12** both retain an acetyl group, highlighting the significant effect of the presence of an acetyl moiety. This suggests that an acetyl group increases binding affinity, probably through some additional interactions with the enzyme. In contrast, compound **14**, which has sulfamate groups at both positions C-16 and C-18, showed only minimal inhibition (<5%). A probable reason for this could be that the simultaneous presence of sulfamate groups at both positions could lead to steric hindrance or unfavorable interactions. Compound **12**, displaying a notable 94.5 % inhibition, reveals again an enhancing effect between acetyl group at position C-18, and the sulfamate group at position C-16. Compound **13**, holding an acetyl group at position C-16 and a sulfamate moiety at C-18 exhibited inhibition of 73.5 %, thus emphasizing the nuanced impact of individual modifications at distinct positions. Compound **12**, with a remarkable inhibition of 94.5 %, again shows a potentiating effect between the acetyl group at position C-18 and the sulfamate group at position C-16.

Some molecular modeling calculations were performed, and Fig. 1 shows the main interactions of compounds **11**, **12** and **15** in 2D representation; for **11** a 3D representation is shown, too. Thereby, the protein structure was prepared and validated using the standard MOE tool. To validate the docking's accuracy, the co-crystallized ligand was redocked



Scheme 3. Reactions and conditions: a) DCM, Ac_2O, DMAP (cat.), 20 °C, 1 day: 10: (41 %), 11 (48 %); b) pyridine, DMAP (cat.), succinic anhydride, microwaves, 200 °C, 3 h, 42 %; c) THF, NMM, ethyl chloroformate, 20 °C, 15 min, then 3, reflux, 48 h, 61 %.

into the enzyme binding site and compared with the crystallographic pose. Additionally, experimental results and known binding mechanisms of comparable inhibitors were utilized to verify the docking parameters.

For the most active compounds, i.e., **11**, **12** and **15**, extra kinetic measurements were performed showing all of them as competitive inhibitors for CA II. Table 3 summarizes these results, and the corresponding Dixon-plots are depicted in Fig. 2.

3. Conclusion

The diterpene isosteviol can be obtained by hydrolysis from stevioside which is a renewable resource and therefore of special interest for synthesis. Inhibitors of the enzyme carbonic anhydrase II are currently in the focus of increasing scientific research because, in addition to their use in the treatment of glaucoma, their use to reduce the side effects of antibody therapies for Alzheimer's disease has recently been discussed. The central structural feature of many CA II inhibitors is the presence of a sulfonamide or a sulfamate group. Isosteviol was therefore converted into (un)-spacered sulfamates, and their ability to act as CA II inhibitors was investigated. Three derivatives, in particular, were found to be effective inhibitors. A conjugate of isosteviol carrying a 5-aminosulfonyl-1,3,4-thiadiazol-2-yl-amino substituent acetylated at C-16 of the isosteviol skeleton and spacered with a succinoyl ester inhibited CA II almost completely as a competitive inhibitor showing a $K_i = 2.59$ μM.

4. Experimental

4.1. General

General information is provided in the supplementary material file.

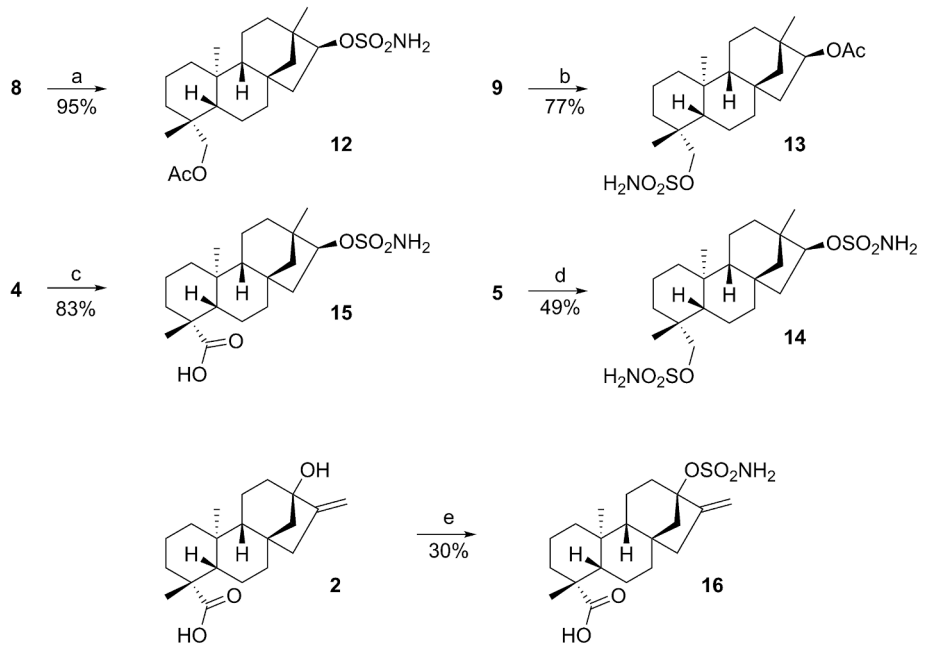
4.2. Syntheses

4.2.1. (4α, 8β, 13β) 13-Methyl-16-oxo-17-norkauran-18-oic acid (1, isosteviol)

Hydrolysis of stevioside (86.0 g, 0.10 mol) in MeOH (500 mL) with aq. HCl (33 %, 90 mL) under reflux for 2 h followed by stirring at 21 °C continued overnight, addition of water (1200 mL), filtration, drying and re-crystallization of the filter cake from EtOH (300 mL) gave **1** (21.6 g, 63 %) as a colorless solid; $R_f = 0.71$ (SiO_2, $CHCl_3$/MeOH 9:1); m.p. = 229–231 °C [lit.: [70] 228–230 °C]; $[\alpha]_D^{20} = -84.02°$ ($c = 0.15$, $CHCl_3$) [lit.: [71] $[\alpha]_D^{20} = -79.3°$ (EtOH)]; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* (%) = 317.0 (100 %, $[M-H]^-$).

4.2.2. (4α) 13-Hydroxy-kaur-16-en-18-oic acid (2, steviol)

Reaction of stevioside (100.0 g, 0.12 mol) in dist. water (8.0 L) with $NaIO_4$ (160.0 g, 0.75 mol) as previously described followed by



Scheme 4. Reactions and conditions: a) DMA, sulfamoyl chloride, 1 day, 21 °C, 95 %; b) DMA, sulfamoyl chloride, 1 day, 21 °C, 77 %; c) DMA, sulfamoyl chloride, 1 day, 21 °C, 49 %; d) DMA, sulfamoyl chloride, 1 day, 21 °C, 83 %; e) DMA, sulfamoyl chloride, 1 day, 21 °C, 30 %.

Table 2
Inhibition of CA II; experiments were performed in triplicate; concentration of the inhibitor 10 μM; AAZ was used as a positive standard.

Compound	Inhibition [%]
7	24.2 ± 1.1
11	99.9 ± 0.1
12	94.5 ± 0.8
13	73.5 ± 1.4
14	< 5
15	94.6 ± 0.2
16	< 5
AAZ	99.9 ± 0.1

crystallization from MeOH gave **2** (10.5 g, 26 %) as a colorless solid; R_f = 0.47 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. = 205 °C [lit.: [71] 212–213 °C]; $[\alpha]_D^{20} = -62.88°$ ($c = 0.09$, $CHCl_3$) [lit.: [72] $[\alpha]_D^{20} = -55.2°$ ($CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 317.2 (100 %, $[M-H]^-$).

*4.2.3. 5-Amino-1,3,4-thiadiazole-2-sulfonamide (**3**)*

Hydrolysis of acetazolamide (**AAZ**, 9.0 g, 40.7 mmol) in conc. HCl (60 mL) for 3 h as previously described gave **3** (6.9 g, 94 %) as a white solid; m.p. 195 °C decomp. (lit.: [73] 215–216 °C); R_F = 0.3 (SiO_2, $CHCl_3$/MeOH, 9:1); MS (ESI, MeOH): *m/z* = 179.0 (100 %, $[M-H]^-$).

*4.2.4. (4α, 8β, 13β, 16β) 16-Hydroxystachan-18-oic acid (**4**)*

A solution of isosteviol **1** (0.5 g, 1.6 mmol) and sodium borohydride (0.09 g, 2.4 mmol) in dry ethanol (20 mL) was stirred at 0 °C for 1 h. The volatiles were removed under reduced pressure, and the residue was extracted with $CHCl_3$ and H_2O. The combined organic layers were washed with brine, and dried ($MgSO_4$) to afford **4** (0.49 g, 97 %) as a colorless solid; R_f = 0.58 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 185–186 °C (lit.: [70] 169 °C); $[\alpha]_D^{20} = -59.33°$ ($c = 0.098$, MeOH); IR (ATR): *ν* = 3472 *m*, 2947 *m*, 2894 *m*, 2836 m, 1701 *m*, 1649 *m*, 1470*w*, 1451*w*, 1386*w*, 1371*w*, 1324*w*, 1290*w*, 1265*w*, 1235*w*, 1187*w*, 1153*w*, 1120*w*, 1070*w*, 1055*w*, 1025*w*, 997*w*, 974*w*, 908*w*, 851*w*, 786*w*, 769*w*, 761*w*, 736*w*, 703*w*, 619*w*, 579*w*, 533*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): *δ* = 3.86 (*dd*, *J* = 10.7, 4.6 Hz, 1H, 16-H), 2.12 (*d*, *J* = 13.4 Hz, 1H, 3-H), 1.93–1.67 (*m*, 6H, 2-H_a, 15-H_a, 6-H_a, 12-H_a, 1-H_a, 15-H_b), 1.65–1.47 (*m*, 4H, 11-H, 6-H_b, 7-H_a), 1.45–1.33 (*m*, 1H, 2-H_b), 1.33–1.24 (*m*, 2H, 7-H_b, 14-H_a), 1.22 (*s*, 4H, 12-H_b, 19-H), 1.09–0.94 (*m*, 4H, 3-H_b, 14-H_b, 5-H, 9-H), 0.90 (*s*, 3H, 17-H), 0.88–0.86 (*m*, 1H, 1-H_b), 0.83 (*s*, 3H, 20-H); ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 183.6 (C-18), 80.6 (C-16), 57.2 (C-5), 56.0 (C-9), 55.4 (C-14), 43.7 (C-4), 42.8 (C-15), 42.3 (C-13), 42.1 (C-8), 41.8 (C-7), 39.9 (C-1), 38.4 (C-10), 37.9 (C-3), 33.9 (C-12), 29.1 (C-19), 25.0 (C-17), 21.9 (C-6), 20.6 (C-11), 19.0 (C-2), 13.3 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 318.9 (90 %, $[M-H]^-$); analysis calcd for $C_{20}H_{32}O_3$ (320.47): C 74.96, H 10.07; found: 74.71, H 10.28.

*4.2.5. (4α, 8β, 13β, 16β) Stachane-16,18-diol (**5**)*

To a solution of **1** (5.0 g, 0.016 mol) in dry THF (50 mL), $LiAlH_4$ (5.0 g, 0.13 mol) was slowly added at ambient temperature followed by heating under reflux for 3 h. Usual aq. work-up followed by chromatography (silica gel, $CHCl_3$/MeOH, 95:5) gave **5** (4.2 g, 88 %) as a white solid; R_f = 0.45 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 156–157 °C; $[\alpha]_D^{20} = -25.08°$ (c = 0.167, MeOH); IR (ATR): *ν* = 3305*br*, 2929 *m*, 2891*w*, 2867*w*, 2838 *m*, 1480*w*, 1454*w*, 1446*w*, 1385*w*, 1371*w*, 1327*w*, 1210*w*, 1157*w*, 1123*w*, 1083*w*, 1062 *m*, 1025 *m*, 999*w*, 970*w*, 958*w*, 930*w*, 840*w*, 759*w*, 704*w*, 626*w*, 535*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* = 3.84 (*dd*, *J* = 10.9, 4.5 Hz, 1H, 16-H), 3.75 (*d*, *J* = 11.0 Hz, 1H, 18-H_a), 3.41 (*d*, *J* = 11.1 Hz, 1H, 18-H_b), 1.86–1.71 (*m*, 3H, 15-H_a, 3-H_a, 12-H_a), 1.71–1.63 (*m*, 2H, 15-H_a, 1-H_a), 1.61–1.45 (*m*, 5H, 6-H_a, 11-H, 2-H_a, 7-H_a), 1.42–1.15 (*m*, 5H, 2-H_b, 7-H_b, 14-H_a, 12-H_b, 6-H_b), 1.05–0.99 (*m*, 2H, 14-H_b, 9-H), 0.99–0.96 (*m*, 1H, 5-H), 0.95 (*s*, 3H, 19-H), 0.94–0.92 (*m*, 1H, 3-H_b), 0.90 (*s*, 3H, 17-H), 0.88 (*s*, 3H, 20-H), 0.86–0.80 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 80.8 (C-16), 65.8 (C-18), 57.1 (C-5), 56.8 (C-9), 55.6 (C-14), 43.1 (C-15), 42.2 (C-13), 42.1 (C-7, C-8), 39.7 (C-1), 38.6 (C-4), 37.8 (C-10), 35.7 (C-3), 33.8 (C-12), 27.3 (C-19), 25.1 (C-17), 20.4 (C-6), 20.4 (C-11), 18.2 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 345.5 (50 %, $[M+K]^+$); analysis calcd for $C_{20}H_{34}O_2$ (306.49): C 78.38, H 11.18; found: C 78.11, H 11.34.

*4.2.6. (4α, 8β, 13β, 16β) 4-[(16-Hydroxy-stachan-18-oyl)oxy]-4-oxobutanoic acid (**6**)*

To a solution of **5** (0.6 g, 1.96 mmol) in dry pyridine (10 mL), DMAP (cat.) and succinic anhydride (0.55 g, 5.5 mmol) were added. The reaction mixture was stirred at 200 °C (microwave assisted; Anton-Paar

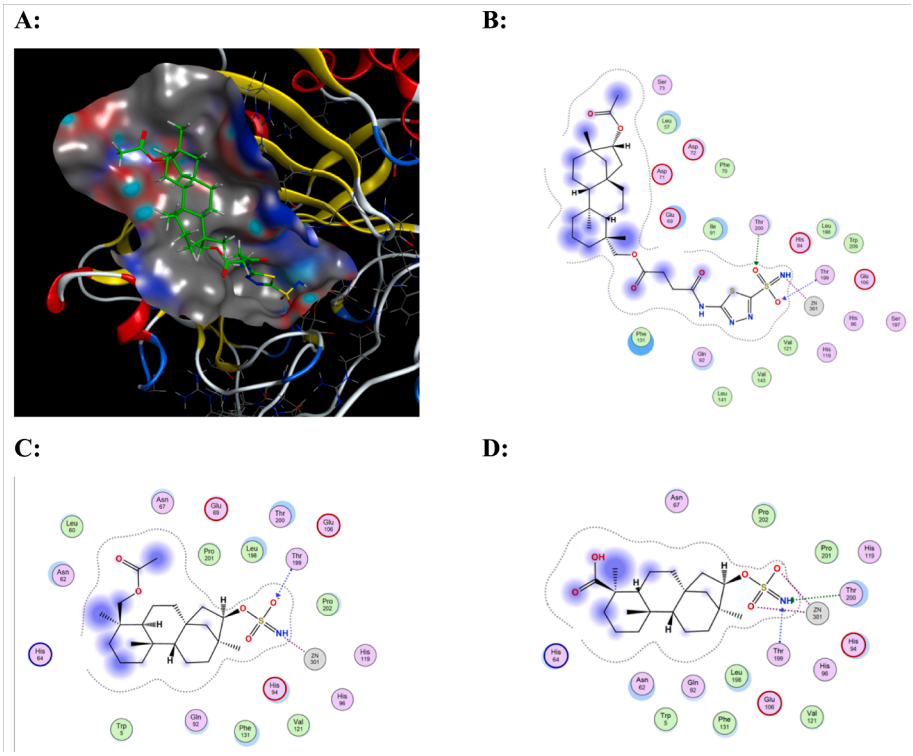


Fig. 1. Calculated preferred localization and orientation of compounds 11, 12 and 15 and CA II: A: 3D modeling results for 11; B: 2D modeling result for 11; C: 2D modeling result for 12; D: 2D modeling result for 15.

Table 3
Determination of inhibition-% and K_i values (in μM) for compounds 11, 12 and 15.

Compound	11	12	15
K_i [μM]	2.59 ± 0.12	3.60 ± 0.17	4.10 ± 0.28
Inhibition-%	99.9 ± 0.1	94.5 ± 0.8	94.6 ± 0.2

GmbH, Monowave) for 3 h. Usual aqueous work up and chromatography (SiO_2, hexanes/ethyl acetate, 7:3) gave **6** (0.552 g, 69 %) as a colorless solid; R_f = 0.23 (SiO_2, hexanes/ethyl acetate, 5:5); m.p. 149–150 °C; $[\alpha]_D^{20}$ = –20.13° (*c* = 0.141, MeOH); IR (ATR): *ν* = 2930*w*, 2841*w*, 1706 *m*, 1455*w*, 1394*w*, 1373*w*, 1320*w*, 1275*w*, 1189*w*, 1165*w*, 1069*w*, 1001*w*, 970*w*, 834*w*, 759*w*, 637*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* = 4.27 (*d*, *J* = 11.0 Hz, 1H, 18-H_a), 3.89 (*d*, *J* = 11.0 Hz, 1H, 18-H_b), 3.85 (*dd*, *J* = 10.8, 4.6 Hz, 1H, 16-H), 2.70–2.58 (*m*, 4H, 22-H, 23-H), 1.86–1.78 (*m*, 1H, 15-H_a), 1.79–1.71 (*m*, 1H, 12-H_a), 1.71–1.63 (*m*, 3H, 3-H_a, 1-H_a, 15-H_b), 1.62–1.46 (*m*, 5H, 6-H_a, 11-H, 2-H_a, 7-H_a), 1.42–1.15 (*m*, 5H, 2-H_b, 7-H_b, 14-H_a, 6-H_b, 12-H_b), 1.06–0.99 (*m*, 2H, 14-H_b, 9-H), 0.99–0.94 (*m*, 2H, 3-H_b, 5-H), 0.93 (*s*, 3H, 19-H), 0.90 (*s*, 6H, 17-H, 20-H), 0.84 (*td*, *J* = 13.1, 4.1 Hz, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 177.4 (C-24), 172.5 (C-21), 80.8 (C-16), 67.9 (C-18), 57.0 (C-5), 56.7 (C-9), 55.6 (C-14), 43.0 (C-15), 42.2 (C-8, C-13), 42.0 (C-7), 39.6 (C-1), 37.7 (C-10), 37.1 (C-4), 36.4 (C-3), 33.8 (C-12), 29.2 (C-22), 29.1 (C-23), 27.7 (C-19), 25.1 (C-17), 20.4 (C-6), 20.4 (C-11), 18.1 (C-2), 15.5 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 429.4 (95 %, $[M + Na]^+$); analysis calcd for $C_{24}H_{38}O_5$ (406.56): C 70.90, H 9.42; found: C 70.68, H 9.63.

4.2.7. (4α, 8β, 13β, 16β) 16-Hydroxystan-18-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate (7)

Compound **6** (0.32 g, 0.79 mmol) was dissolved in dry THF (50 mL),

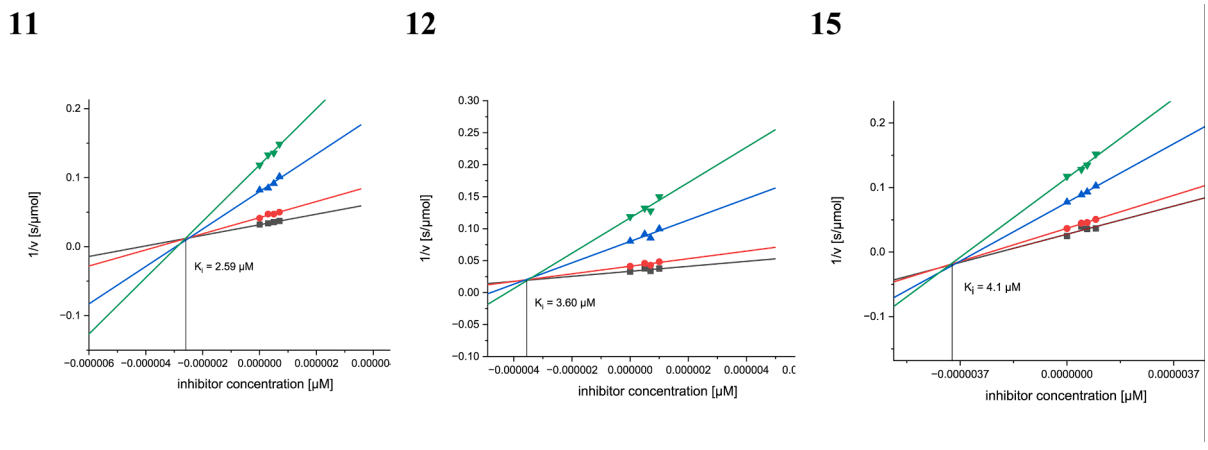


Fig. 2. Dixon plots for compounds 11, 12 and 15.

4-methylmorpholine (0.16 g, 1.58 mmol) and ethyl chloroformate (0.171 g, 1.58 mmol) were added. The reaction mixture was stirred at 20 °C for 15 min. Compound **3** (0.215 g, 1.2 mmol) was added, and the mixture was heated under reflux for another 48 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with aq. NaOH (2M), water and brine and dried ($MgSO_4$). Chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **7** (0.232 g, 52 %) as a white solid; $R_f = 0.44$ (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 126–127 °C; $[\alpha]_D^{20} = -12.16°$ ($c = 0.06$, MeOH); UV–Vis (MeOH): λ_{max} (log ε) = 265 nm (3.81); IR (ATR): ν = 3502*br*, 3286*br*, 2919 *m*, 2850 *m*, 1685 *m*, 1518 *m*, 1451*w*, 1419*w*, 1345 *m*, 1325 *m*, 1291*w*, 1221*w*, 1170 *m*, 1156 *m*, 1088*w*, 1051*w*, 1000*w*, 973*w*, 952*w*, 926*w*, 797*w*, 756*w*, 627*w*, 587*w*, 510*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.31 (*s*, 3H, NH, NH_2), 4.46 (*d*, J = 4.1 Hz, 1H, OH), 4.24 (*d*, J = 10.9 Hz, 1H, 18-H_a), 3.74 (*d*, J = 11.0 Hz, 1H, 18-H_b), 3.65 (*dt*, J = 9.5, 4.1 Hz, 1H, 16-H), 2.84–2.77 (*m*, 2H, 23-H), 2.71–2.64 (*m*, 2H, 22-H), 1.72–1.64 (*m*, 2H, 15-H_a, 12-H_a), 1.63–1.33 (*m*, 8H, 6-H_a, 1-H_a, 3-H_a, 11-H_a, 15-H_b, 2-H_a, 11-H_b, 7-H_a), 1.33–1.22 (*m*, 2H, 2-H_b, 7-H_b), 1.22–1.10 (*m*, 2H, 14-H_a, 6-H_b), 1.10–0.99 (*m*, 1H, 12-H_b), 0.99–0.87 (*m*, 4H, 3-H_b, 14-H_b, 5-H, 9-H), 0.85 (*s*, 3H, 19-H), 0.83 (*s*, 3H, 20-H), 0.81 (*s*, 3H, 17-H), 0.80–0.71 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.8 (C-21), 171.2 (C-24), 164.3 (C-26), 161.0 (C-25), 78.4 (C-16), 66.4 (C-18), 62.8, 56.2 (C-5), 56.2 (C-9), 55.1 (C-14), 42.7 (C-15), 41.6 (C-8), 41.6 (C-13), 41.5 (C-7), 38.9 (C-1), 37.1 (C-10), 36.7 (C-4), 35.6 (C-3), 33.6 (C-12), 29.9 (C-23), 28.3 (C-22), 27.2 (C-19), 25.0 (C-17), 19.7 (C-11), 19.6 (C-6), 17.5 (C-2), 15.1 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 567.4 (95 %, $[M-H]^-$); analysis calcd for $C_{26}H_{40}N_4O_6S_2$ (568.75): C 54.91, H 7.09, N 9.85; found: C 54.77, H 7.30, N 9.63.

*4.2.8. (4α, 8β, 13β, 16β) 16-Hydroxystachan-18-yl acetate (**8**) and (4α, 8β, 13β, 16β) 18-hydroxystach-16-yl acetate (**9**)*

To a solution of **5** (2.0 g, 6.6 mmol) in dry DCM, acetic anhydride (2 g, 40 mmol), triethylamine (4.0 g, 19.8 mmol), and DMAP (cat.) were added, and the mixture was stirred at 20 °C for 1 day. Usual aqueous work-up followed by column chromatography (SiO_2, hexanes/ethyl acetate, 8:2) gave **8** (0.931 g, 41 %) and **9** (1.08 g, 48 %) each as a white solid.

Data for **8**: R_f = 0.89 (SiO_2, hexanes/ethyl acetate, 6:4); m.p. 149–150 °C; $[\alpha]_D^{20} = -26.13°$ ($c = 0.169$, MeOH); IR (ATR): ν = 3526 *m*, 3499*w*, 2963*w*, 2932 *m*, 2903 *m*, 2840 *m*, 1720 *s*, 1481*w*, 1444*w*, 1393 *m*, 1371 *m*, 1319*w*, 1259 *s*, 1209*w*, 1125*w*, 1089*w*, 1052*w*, 1046 *m*, 1031 *m*, 1001*w*, 982 *m*, 913*w*, 855*w*, 773*w*, 738*w*, 704*w*, 626*w*, 609*w*, 511*br*, 465*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.23 (*d*, J = 11.0 Hz, 1H, 18-H_a), 3.84 (*d*, J = 10.9 Hz, 2H, 16-H, 18-H_b), 2.03 (*s*, 3H, 22-H), 1.83 (*ddd*, J = 14.2, 4.4, 3.1 Hz, 1H, 15-H_a), 1.79–1.71 (*m*, 1H, 12-H_a), 1.72–1.64 (*m*, 3H, 3-H_a, 1-Ha, 15-H_b), 1.62–1.53 (*m*, 3H, 6-H_a, 11-H), 1.53–1.46 (*m*, 2H, 2-H_a, 7-H_a), 1.41–1.34 (*m*, 2H, 2-H_b, 7-H_b), 1.29 (*m*, 1H, 14-H_a), 1.25–1.17 (*m*, 2H, 6-H_b, 12-H_b), 1.06–0.98 (*m*, 2H, 14-H_b, 9-H), 0.99–0.94 (*m*, 2H, 3-H_b, 5-H), 0.93 (*s*, 3H, 19-H), 0.91 (*s*, 3H, 20-H), 0.90 (*s*, 3H, 17-H), 0.84 (*td*, J = 13.3, 4.0 Hz, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.5 (C-21), 80.7 (C-16), 67.3 (C-18), 57.0 (C-5), 56.8 (C-9), 55.6 (C-14), 43.1 (C-15), 42.2 (C-13), 42.2 (C-8), 42.0 (C-7), 39.6 (C-1), 37.7 (C-10), 37.1 (C-4), 36.3 (C-3), 33.8 (C-12), 27.7 (C-19), 25.1 (C-17), 21.1 (C-22), 20.4 (C-11), 20.3 (C-6), 18.1 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 371.5 (45 %, $[M + Na]^+$); analysis calcd for $C_{22}H_{36}O_3$ (348.53): C 75.82, H 10.41; found: C 75.61, H 10.55.

Data for **9**: R_f = 0.85 (SiO_2, hexanes/ethyl acetate, 6:4); m.p. 163–165 °C; $[\alpha]_D^{20} = -40.49°$ ($c = 0.118$, MeOH); IR (ATR): ν = 3529 *m*, 2994*w*, 2928 *m*, 2871*w*, 2845 *m*, 1712 *s*, 1467*w*, 1451*w*, 1437*w*, 1386 *m*, 1370 *m*, 1258 *s*, 1207*w*, 1153*w*, 1119*w*, 1057 *m*, 1034 *s*, 974 *m*, 909*w*, 851*w*, 762*w*, 714*w*, 631*w*, 608*w*, 502*br* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.72 (*dd*, J = 8.7, 6.4 Hz, 1H, 16-H), 3.74 (*d*, J = 10.9 Hz, 1H, 18-H_a), 3.39 (*d*, J = 10.1 Hz, 1H, 18-H_b), 2.06 (*s*, 3H, 22-H), 1.83–1.74 (*m*, 4H, 15-H, 3-H_a, 12-H_a), 1.70–1.62 (*m*, 1H, 1-H_a), 1.61–1.46 (*m*, 5H, 6-H_a, 11-H, 2-H_a, 7-H_a), 1.42–1.29 (*m*, 3H, 2-H_b, 7-H_b, 14-H_a), 1.29–1.12 (*m*, 2H, 12-H_b, 6-H_b), 1.08–1.01 (*m*, 2H, 14-H_b, 9-H), 0.99–0.96 (*m*, 1H, 5-H), 0.95 (*s*, 3H, 19-H), 0.94–0.91 (*m*, 1H, 3-H_b), 0.90 (*s*, 3H, 17-H), 0.88–0.86 (*m*, 1H, 1-H_b), 0.85 (*s*, 3H, 20-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.5 (C-21), 81.8 (C-16), 65.7 (C-18), 57.1 (C-5), 56.7 (C-9), 55.2 (C-14), 42.4 (C-8), 41.9 (C-13), 41.6 (C-7), 40.9 (C-15), 39.7 (C-1), 38.6 (C-4), 37.8 (C-10), 35.6 (C-3), 34.7 (C-12), 27.2 (C-19), 25.1 (C-17), 21.3 (C-22), 20.3 (C-11), 20.2 (C-6), 18.1 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 371.3 (40 %, $[M + Na]^+$); analysis calcd for $C_{22}H_{36}O_3$ (348.53): C 75.82, H 10.41; found: C 77.59, H 10.62.

*4.2.9. 4-{[(4α, 8β, 13β, 16β) 16-(Acetyloxy)stachan-18-yl]oxy}-4-oxobutanoic acid (**10**)*

To a solution of **9** (0.542 g, 1.55 mmol) in dry pyridine (10 mL), DMAP (cat.) and succinic anhydride (0.311 g, 3.11 mmol) were added. The reaction mixture was stirred (microwave, as above) at 200 °C for 3 h. Usual aqueous work up and chromatography (SiO_2, hexanes/ethyl acetate, 7:3) gave **10** (0.292 g, 42 %) as a colorless solid; R_f = 0.14 (SiO_2, hexanes/ethyl acetate, 7:3); m.p. 100–102 °C; $[\alpha]_D^{20} = -26.6°$ ($c = 0.114$, MeOH); IR (ATR): ν = 2927 *m*, 2848 *m*, 1733 *s*, 1712 *s*, 1455 *m*, 1439 *m*, 1372 *m*, 1241 *s*, 1159 *s*, 1057 *m*, 1035 *m*, 995*w*, 973*w*, 938*w*, 839*w*, 754 *m*, 629*w*, 605 *m*, 550*br*, 484*w*, 456*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.76–4.69 (*m*, 1H, 16-H), 4.28 (*d*, J = 11.0 Hz, 1H, 18-H_a), 3.86 (*d*, J = 10.5 Hz, 1H, 18-H_b), 2.71–2.57 (*m*, 4H, 24-H, 25-H), 2.06 (*s*, 3H, 22-H), 1.82–1.72 (*m*, 2H, 15-H, 12-H_a), 1.71–1.61 (*m*, 2H, 1-H_a, 3-H_a), 1.61–1.45 (*m*, 5H, 11-H, 6-H_a, 2-H_a, 7-H_a), 1.42–1.29 (*m*, 3H, 2-H_b, 7-H_b, 14-H_a), 1.29–1.18 (*m*, 2H, 6-H_b, 12-H_b), 1.10–0.94 (*m*, 4H, 14-H_b, 9-H, 5-H, 3-H_b), 0.93–0.91 (*m*, 3H, 19-H), 0.89 (*s*, 3H, 17-H), 0.87 (*s*, 3H, 20-H), 0.85–0.79 (*m*, 1H, 1-H_b); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.8 (C-26), 172.4 (C-23), 171.6 (C-21), 81.7 (C-16), 67.8 (C-18), 57.0 (C-5), 56.6 (C-9), 55.2 (C-14), 42.4 (C-8), 41.7 (C-13), 41.6 (C-7), 40.8 (C-15), 39.5 (C-1), 37.7 (C-4), 37.1 (C-10), 36.4 (C-3), 34.6 (C-12), 29.1 (C-25), 29.1 (C-24), 27.7 (C-19), 25.0 (C-17), 21.3 (C-22), 20.3 (C-11), 20.2 (C-6), 18.0 (C-2), 15.5 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 447.6 (85 %, $[M-H]^-$); analysis calcd for $C_{26}H_{40}O_6$ (448.60): C 69.61, H 8.99; found: C 69.45, H 9.14.

*4.2.10. (4α, 8β, 13β, 16β) 18-(Acetyloxy)-stachan-16-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate (**11**)*

Compound **10** (0.371 g, 0.83 mmol) was dissolved in dry THF (50 mL), 4-methylmorpholine (0.167 g, 1.65 mmol) and ethyl chloroformate (0.179 g, 1.65 mmol.) were added. The reaction mixture was stirred at 20 °C for 15 min. Compound **3** (0.18 g, 1 mmol) was added, and the mixture was heated under reflux for another 48 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with aq. NaOH (2M), water and brine and dried ($MgSO_4$). Chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **11** (0.31 g, 61 %) as a white solid; R_f = 0.62 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 135–136 °C; $[\alpha]_D^{20} = -27.17°$ ($c = 0.069$, MeOH); UV–Vis (MeOH): λ_{max} (log ε) = 265 nm (3.96); IR (ATR): ν = 3253*br*, 2927*br*, 2847*w*, 1705 *m*, 1527 *m*, 1432*br*, 1370 *m*, 1243 *m*, 1173 *m*, 1086*w*, 1057*w*, 1034*w*, 973*w*, 912*w*, 754*w*, 655*w*, 605 *m*, 508*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.31 (*s*, 2H, NH_2), 4.66 (*t*, J = 7.5 Hz, 1H, 16-H), 4.23 (*d*, J = 11.0 Hz, 1H, 18-H_a), 3.74 (*d*, J = 11.0 Hz, 1H, 18-H_b), 2.83–2.75 (*m*, 2H, 25-H), 2.72–2.63 (*m*, 2H, 24-H), 2.01 (*s*, 3H, 22-H), 1.74–1.64 (*m*, 3H, 15-H, 12-H_a), 1.63–1.40 (*m*, 6H, 1-H_a, 3-H_a, 11-H, 6-H_a, 7-H_a), 1.37–1.10 (*m*, 5H, 7-H_b, 14-H_a, 2-H_b, 12-H_b, 6-H_b), 1.09–0.98 (*m*, 2H, 14-H_b, 9-H), 0.97–0.88 (*m*, 2H, 5-H, 3-H_b), 0.86 (*s*, 3H, 19-H), 0.85 (*s*, 3H, 17-H), 0.81 (*s*, 3H, 20-H), 0.78–0.76 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.8 (C-23), 171.5 (C-26), 170.5 (C-21), 164.0 (C-28), 161.5 (C-27), 80.6 (C-16), 66.3 (C-18), 56.0 (C-5), 55.7 (C-9), 54.3 (C-14), 41.7 (C-8), 41.0 (C-13), 40.9 (C-7), 40.2 (C-15), 38.8 (C-1), 35.6 (C-3), 34.0 (C-12), 30.1 (C-25), 28.4 (C-24), 27.2 (C-19), 24.7 (C-17), 20.9 (C-22), 19.6 (C-11), 19.6 (C-6), 17.4 (C-2), 15.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 609.9 (95 %,

[M−H]$^-$); analysis calcd for $C_{28}H_{42}N_4O_7S_2$ (610.79): C 55.06, H 6.93, N 9.17; found: C 54.81, H 7.20, N 9.34.

4.2.11. (4α, 8β, 13β, 16β) 18-[(Aminosulfonyl)oxy]stachan-16-yl acetate (12)

Compound **8** (0.14 g, 0.4 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.116 g, 1.0 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent evaporated under reduced pressure; column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:1) gave **12** (0.163 g, 95 %) as a colorless solid; R_f = 0.9 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m.p. 171–173 °C; $[\alpha]_D^{20}$ = –34.40° (*c* = 0.121, MeOH); IR (ATR): *ν* = 3344 *m*, 3239*w*, 2964*w*, 2946*w*, 2918 *m*, 2883*w*, 2842 *m*, 1700 *s*, 1557*w*, 1462 *m*, 1442*w*, 1373 *s*, 1298*w*, 1271*w*, 1247*w*, 1221*w*, 1180 *s*, 1126*w*, 1030 *m*, 981 *m*, 953 *m*, 915 *m*, 884*w*, 861 *s*, 817 *m*, 788 *m*, 735*w*, 712*w*, 611*w*, 590*w*, 562*w*, 544 *m*, 518*w*, 479*w*, 456*w*, 431*w*, 423 *m* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 7.34 (*s*, 2H, NH_2), 4.41 (*dd*, *J* = 10.7, 3.8 Hz, 1H, 16-H), 4.22 (*d*, *J* = 11.0 Hz, 1H, 18-H_a), 3.77 (*d*, *J* = 11.0 Hz, 1H, 18-H_b), 2.07 (*dt*, *J* = 14.6, 3.2 Hz, 1H, 15-H_a), 1.99 (*s*, 3H, 22-H), 1.76–1.54 (*m*, 5H, 15-H_b, 1-H_a, 3-H_a, 12-H_a, 11-H_a), 1.53–1.42 (*m*, 4H, 2-H_a, 11-H_b, 6-H_a, 7-H_a), 1.39–1.26 (*m*, 2H, 7-H_b, 2-H_b), 1.25–1.14 (*m*, 3H, 14-H_a, 6-H_b, 12-H_b), 1.11–1.00 (*m*, 2H, 14-H_b, 9-H), 1.01–0.94 (*m*, 2H, 3-H_b, 5-H), 0.92 (*s*, 3H, 17-H), 0.90 (*s*, 3H, 19-H), 0.86 (*s*, 3H, 20-H), 0.85–0.78 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): *δ* = 170.4 (C-21), 86.4 (C-16), 66.0 (C-18), 56.0 (C-5), 55.6 (C-9), 53.8 (C-14), 41.7 (C-8), 41.6 (C-13), 41.0 (C-7), 39.9 (C-15), 38.8 (C-1), 37.0 (C-10), 36.6 (C-4), 35.7 (C-3), 33.7 (C-12), 27.3 (C-19), 24.1 (C-17), 20.7 (C-24), 19.7 (C-11), 19.6 (C-6), 17.5 (C-2), 15.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 426.7 (95 %, [M−H]$^-$); analysis calcd for $C_{22}H_{37}NO_5S$ (427.60): C 61.8, H 8.72, N 3.28; found: C 61.56, H 7.96, N 2.99.

4.2.12. (4α, 8β, 13β, 16β) 16-[(Aminosulfonyl)oxy]stachan-18-yl acetate (13)

Compound **9** (0.2 g, 0.58 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.134 g, 1.16 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure and the column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:1) gave **13** (0.19 g, 77 %) as a colorless solid; R_f = 0.93 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m.p. 175–177 °C; $[\alpha]_D^{20}$ = −37.88° (*c* = 0.103, MeOH); IR (ATR): *ν* = 3272*br*, 3115*br*, 2928 *m*, 2846 *m*, 1715 *m*, 1630*w*, 1563*w*, 1455*w*, 1371 *m*, 1260 *m*, 1182 *m*, 1122*w*, 1057*w*, 1035*w*, 969 *m*, 924 *m*, 836 *m*, 754*w*, 715*w*, 676*w*, 611*w*, 552*w*, 503*w*, 483*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 4.75–4.69 (*m*, 3H, NH_2, 16-H), 4.35 (*d*, *J* = 9.3 Hz, 1H, 18-H_a), 3.95 (*d*, *J* = 9.3 Hz, 1H, 18-H_b), 2.06 (*s*, 3H, 22-H), 1.83–1.75 (*m*, 4H, 3-H_a, 12-H_a, 15-H), 1.72–1.64 (*m*, 1H, 1-H_a), 1.62–1.47 (*m*, 5H, 11-H, 6-H_a, 2-H_a, 7-H_a), 1.46–1.31 (*m*, 3H, 2-H_b, 7-H_b, 14-H_a), 1.30–1.14 (*m*, 2H, 12-H_b, 6-H_b), 1.10–1.02 (*m*, 4H, 14-H_b, 9-H, 5-H, 3-H_b), 1.01 (*s*, 3H, 19-H), 0.90 (*s*, 3H, 17-H), 0.88 (*s*, 3H, 20-H), 0.86–0.82 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 171.6 (C-21), 81.7 (C-16), 74.7 (C-18), 56.9 (C-5), 56.6 (C-9), 55.1 (C-14), 42.3 (C-8), 41.6 (C-7, C-13), 40.9 (C-15), 39.3 (C-1), 37.7 (C-10), 37.3 (C-4), 35.8 (C-3), 34.6 (C-12), 27.4 (C-19), 25.0 (C-17), 21.3 (C-22), 20.3 (C-11), 20.2 (C-6), 17.9 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 426.7 (95 %, [M−H]$^-$); analysis calcd for $C_{22}H_{37}NO_5S$ (427.6): C 61.80, H 8.72, N 3.28; found: C 61.56, H 8.97, N 2.99.

4.2.13. (4α, 8β, 13β, 16β) Stachane-16,18-diyl disulfamate (14)

Compound **5** (0.27 g, 0.88 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.254 g, 2.2 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure and column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:1) gave **14** (0.2 g, 49 %) as a colorless solid; R_f = 0.26 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 167–169 °C; $[\alpha]_D^{20}$ = −30.97° (*c* = 0.128, MeOH); IR (ATR): *ν* = 3399*w*, 3377*br*, 3280 *m*, 2987*w*, 2940 *m*, 2893 *m*, 2845 *m*, 1533*w*, 1456*w*, 1343 *s*, 1183 *s*, 1169 *s*, 1049*w*, 997 *s*, 941 *s*, 914 *s*, 863 *m*, 840 *m*, 798 *m*, 759*w*, 595 *m*, 551 *m*, 488*w*, 473*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 7.36 (*s*, 4H, NH_2), 4.41 (*dd*, *J* = 10.8, 3.8 Hz, 1H, 16-H), 4.11 (*d*, *J* = 9.6 Hz, 1H, 18-H_a), 3.76 (*d*, *J* = 9.6 Hz, 1H, 18-H_b), 2.08 (*dt*, *J* = 14.7, 2.9 Hz, 1H, 15-H_a), 1.77–1.60 (*m*, 4H, 15-H_b, 3-H_a, 12-H_a, 1-H_a), 1.60–1.42 (*m*, 5H, 6-H_a,11-H, 2-H_a, 7-H_a), 1.41–1.11 (*m*, 5H, 2-H_b, 7-H_b, 14-H_a, 12-H_b, 6-H_b), 1.11–0.96 (*m*, 4H, 14-H_b, 9-H, 5-H, 3-H_b), 0.93 (*s*, 6H, 17-H, 19-H), 0.87 (*s*, 3H, 20-H), 0.86–0.79 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): *δ* = 86.4 (C-16), 71.7 (C-18), 55.9 (C-5), 55.5 (C-9), 53.7 (C-14), 41.7 (C-13), 41.6 (C-8), 41.0 (C-7), 39.8 (C-15), 38.7 (C-1), 37.0 (C-10), 36.6 (C-4), 35.4 (C-3), 33.7 (C-12), 27.1 (C-19), 24.1 (C-17), 19.7 (C-11), 19.6 (C-6), 17.4 (C-2), 15.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 463.2 (95 %, [M−H]$^-$) 927.3 (60 %, [2M−H]$^-$); analysis calcd for $C_{20}H_{36}N_2O_6S_2$ (464.64): C 51.70, H 7.81, N 6.03; found: C 51.52, H 8.02, N 5.83.

4.2.14. (4α, 8β, 13β, 16β) 16-[(Aminosulfonyl)oxy]stachan-18-oic acid (15)

Compound **4** (0.27 g, 0.84 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.196 g, 1.7 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 8:2) to yield **15** (0.28 g, 83 %) as a colorless solid; R_f = 0.43 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 155–157 °C; $[\alpha]_D^{20}$ = –60.39° (*c* = 0.149, MeOH); IR (ATR): *ν* = 3345*br*, 3255 *m*, 2932 *m*, 2845*w*, 1692 *m*, 1607 *m*, 1574*w*, 1455 *m*, 1362*w*, 1339*br*, 1264*w*, 1249*w*, 1226 *m*, 1174 *s*, 1159 *m*, 1020*w*, 967*w*, 943*w*, 928*w*, 880*w*, 868*w*, 854*w*, 822*w*, 786*w*, 766*w*, 740*w*, 713*w*, 666*w*, 646*w*, 615*w*, 584*w*, 567*w*, 546*w*, 525*w*, 434*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 11.96 (*s*, 1H, OH), 7.34 (*s*, 2H, NH_2), 4.40 (*dd*, *J* = 10.7, 4.0 Hz, 1H, 16-H), 2.10–1.97 (*m*, 2H, 15-H_a, 3-H_a), 1.82–1.61 (*m*, 5H, 2-H_a, 15-H_b, 6-H_a, 12-H_a, 1-H_a), 1.60–1.41 (*m*, 4H, 6-H_b, 11-H, 7-H_a), 1.37–1.15 (*m*, 4H, 2-H_b, 7-H_b, 14-H_a, 12-H_b), 1.09 (*s*, 3H, 19-H), 1.07–0.96 (*m*, 3H, 14-H_b, 5-H, 9-H), 0.92 (*s*, 4H, 3-H_b, 17-H), 0.85 (*td*, *J* = 13.4, 4.1 Hz, 1H, 1-H_b), 0.77 (*s*, 3H, 20-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): *δ* = 178.6 (C-18), 86.4 (C-16), 56.0 (C-5), 54.9 (C-9), 53.6 (C-14), 42.8 (C-4), 41.8 (C-13), 41.6 (C-8), 40.9 (C-7), 39.8 (C-15), 39.4 (C-1), 37.6 (C-10), 37.6 (C-3), 33.7 (C-12), 28.6 (C-19), 24.1 (C-17), 21.4 (C-6), 19.7 (C-11), 18.6 (C-2), 13.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 398.2 (60 %, [M−H]$^-$) 797.3 (90 %, [2M−H]$^-$); analysis calcd for $C_{20}H_{33}NO_5S$ (399.55): C 60.12, H 8.33, N 3.51; found: C 59.96, H 8.55, N 3.33.

4.2.15. (4α) 13-(Aminosulfonyl)oxy-kaur-16-en-18-oic acid (16)

Compound **2** (0.27 g, 0.85 mol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.196 g, 1.7 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure, and column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 7:3) gave **16** (0.1 g, 30 %) as a colorless solid; R_f = 0.54 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 121–122 °C; $[\alpha]_D^{20}$ = –33.49° (*c* = 0.067, MeOH); IR (ATR): *ν* = 3380*br*, 3276*br*, 2927*br*, 2849*w*, 1690 *s*, 1556*w*, 1463*w*, 1448*w*, 1363*br*, 1262*w*, 1176 *s*, 1028 *m*, 1000 *m*, 958 *m*, 926 *s*, 904 *m*, 852 *m*, 843 *m*, 799*w*, 755 *m*, 671*w*, 616*w*, 589*w*, 550*w*, 491*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 12.01 (*s*, 1H, COOH), 7.39 (*s*, 2H, NH_2), 4.96 (*brs*, 1H, 17-H_a), 4.87 (*brs*, 1H, 17-H_b), 2.54–2.51 (*m*, 1H, 14-H_a), 2.25–2.14 (*m*, 1H, 3-H_a), 2.11–1.99 (*m*, 3H, 15-H, 12-H_a), 1.82–1.70 (*m*, 6H, 2-H_a, 6-H, 1-H_a, 11-H_a, 14-H_b), 1.63–1.47 (*m*, 3H, 12-H_b, 11-H_b, 7-H_a), 1.46–1.29 (*m*, 2H, 7-H_b, 2-H_b), 1.11 (*s*, 3H, 19-H), 1.06–1.02 (*m*, 1H, 5-H), 1.02–0.92 (*m*, 2H, 9-H, 3-H_b), 0.88 (*s*, 3H, 20-H), 0.79 (*td*, *J* = 13.3, 3.1 Hz, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): *δ* = 178.6 (C-18), 151.1 (C-16), 104.6 (C-17), 91.2 (C-13), 55.7 (C-5), 52.5 (C-9), 45.9 (C-15), 42.8 (C-4), 42.2 (C-14), 42.2 (C-8), 40.6 (C-7), 40.1 (C-1), 38.8

(C-10), 37.5 (C-12), 37.4 (C-3), 28.5 (C-19), 21.5 (C-6), 20.0 (C-11), 18.8 (C-2), 15.2 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 396.3 (60 %, $[M-H]^-$, 793.3 (95 %, $[2M-H]^-$); analysis calcd for $C_{20}H_{31}NO_5S$ (397.53): C 60.43, H 7.86, N 3.52; found: C 60.19, H 8.01, N 3.36.

CRediT authorship contribution statement

Toni C. Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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