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Clarifying the structure of granadaene: Total synthesis of related analogue [2]-granadaene and confirmation of its absolute stereochemistry

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ABSTRACT

Streptococcus agalactiae is an important agent in the infection of neonates in the first world. One of the most extended methods for its identification is based on the detection of a characteristic red pigment in the patient samples, named [12]-granadaene (**1**). In this article, we present a modular and flexible approach to simple analogues of this ornithine rhamno-polyene **1** and the elucidation of the most important features of its structure: the absolute configuration at C-27, the stereochemistry of the anomeric center and the link of the amino acid ornithine to the rest of the structure.

1. Introduction

Hemolytic *Streptococcus agalactiae* (group B streptococcus [GBS]) is the most important bacterium causing life-threatening infections in neonates in the first world,[1] constituting an important cause of death in this population. Nowadays, its detection is mandatory for all pregnant women between 35 and 37 weeks of gestation.[2] In this context, one of the most extended method for GBS identification is based on the detection of a characteristic red pigment in the Granada medium.[3] Despite its interest, little is known about the nature of this key pigment and also unknown about the nature of hemolysin GBS. The GBS hemolysin has yet to be isolated, largely because its activity is unstable. Interestingly, GBS presents the same genes for the synthesis of the red pigment and for the GBS virulence factor hemolysine. The deduced proteins display similarities to ABC (ATP binding cassette) transporters and prokaryotic fatty acid biosynthesis enzymes.[4,5] Until recently, a carotene-type structure was assumed for GBS pigment,[4] but intringuingly, genes coding for carotene biosynthesis are not present in GBS. In 2006, some of us isolated the main component of that highly insoluble red pigment, which was named [12]-granadaene (**1**)[6] and constituted the first example of a glycopolyene isolated from gram-positive bacteria.[7] A structure of ornithine rhamno-polyene was tentatively proposed based on NMR studies in DMSO-d_6/TFA-d mixtures (see Scheme 1) of scarce amounts of compound **1**, owing to the insolubility of the red pigment in any other solvent mixture.[7] Taking into account the relevance of this red pigment in the worldwide detection of GBS in pregnant women and the apparent biogenetic relationship with virulence factor hemolysine, the clarification of some uncertainties in its structure is mandatory. Thus, the absolute configuration at C-27, the configuration in the anomeric center of the sugar moiety, and the link of the amino acid to the rest of the structure, remain unclear.[8] Although the total synthesis of granadaene (**1**) would be essential to determine all these structural unknowns, its biosynthetic pathway and the structure of the hemolytic compound in GBS,[5] its structural complexity could hinder this goal. Thus, the preparation of simple derivatives of granadaene (**1**), including different parts of its structure, would be a good solution to clarify the structure of the pigment. In this article, we have developed an easy, modular approach to the synthesis of granadaene-type compounds (**2**–**4**) (see Scheme 2), which has allowed the determination of the unknown structural aspects for granadaene (**1**).

2. Results and discussion

Before to accomplish the total synthesis of granadaene (**1**), all the structural unknowns of its structure have to be previously clarified. To reach this objective, we decided to prepare simple analogues of **1**, as compounds **2**, **3** and [2]-granadaene **4**, a simple derivative containing only two double bonds of the polyenic chain

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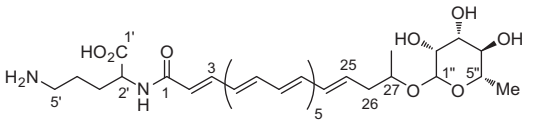
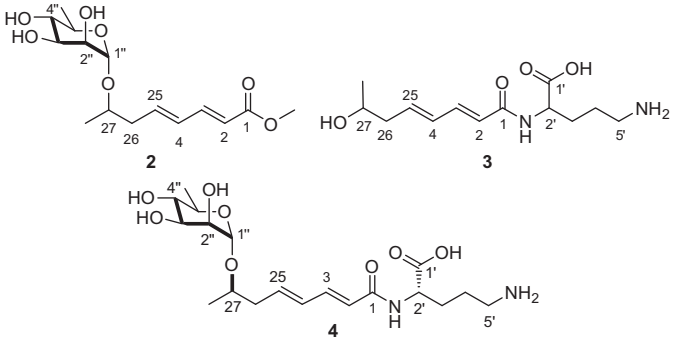


Scheme 1. Proposed structure of natural polyene pigment [12]-granadaene (**1**).



Scheme 2. Simple derivatives of [12]-granadaene (**1**).

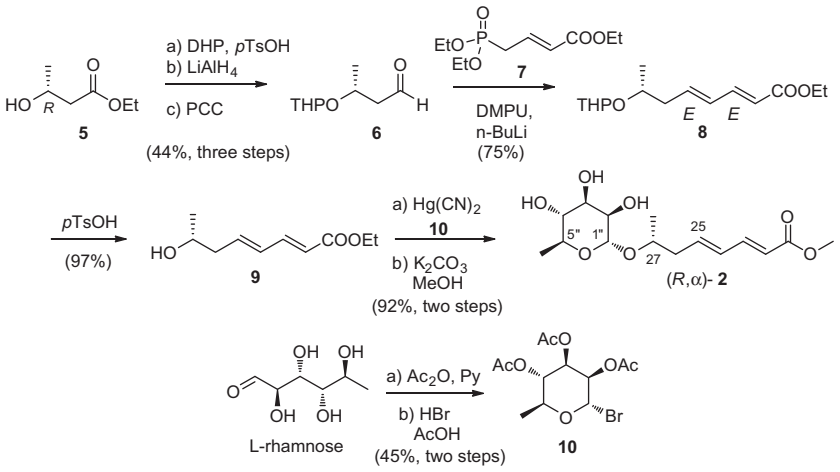
(Scheme 2).[9] The similarity between the chemical environments of such fragments with those proposed in the original structure of **1** should ensure a simple comparison between the corresponding spectroscopic data.

These compounds **2**–**4** contain all the unknown structural aspects in granadaene (**1**) and their synthesis can be accomplished in a few steps. Thus, to determinate the absolute configuration at C-27 and the configuration in the anomeric center of the rhamnose including moiety, we decided to prepare compound **2**, which has a similar chemical environment that present in **1** for such hemisphere of the molecule. The synthesis of **2** is depicted in Scheme 3.

We started from commercial available racemic **5**, which was subsequently transformed in aldehyde **6**[10] in three steps. Horner-Wadsworth-Emmons reaction[11] between aldehyde **6** and the commercial phosphonate **7** yielded diene **8** in good yield and complete *E,E* stereoselectivity. Deprotection of THP group under acidic conditions gave alcohol **9**, which was subsequently used in the glycosylation process. For it, we used the L-bromorhamnose **10**, which was synthesized in only two steps (acetylation and bromination with HBr under acidic conditions) from L-rhamnose. Finally, treatment of alcohol **9** with L-bromorhamnose **10**[12] in the presence of $Hg(CN)_2$ gave exclusively the α anomer of **2**.[13] Subsequent acetoxy group saponification generated a mixture of two diastereoisomers. The NMR analysis of that mixture showed that the ^1H and ^{13}C NMR signals at C-27, C-28 and C-1″ do not overlap and therefore and unambiguous structural assignment can be carried out using the corresponding enantiopure starting materials **5**. When the synthetic sequence was repeated using (*R*)-**5** (see Scheme 3), similar yields were obtained in all the reactions. Finally, the comparison of ^1H and ^{13}C NMR data of (*R*,α)-**2** with those previously described for the corresponding subunit in [12]-granadaene (**1**)[7] (Tables 1 and 2) confirmed the same absolute stereochemistry for compound **1**.[14]

Thus, ^1H and ^{13}C NMR signals corresponding to the anomeric center C-1″ are practically identical in both compounds. Additionally, the NMR signals corresponding to the methyl group at C-27 in **2** are also very close to those previously reported for the natural product **1**. Therefore, the synthesis of compound **2** allowed us to establish unambiguously two key structural characteristics of **1**: the α configuration for the anomeric center in the rhamnose moiety and the *R* configuration at C-27.

Once the structural features of **1** involving the L-rhamnose moiety were revealed, we turned our attention to the hemisphere containing the amino acid subunit. Within this context, the link of the amino acid fragment to the rest of the compound had to be determined. To this end, we accomplished the synthesis of the granadaene derivative **3**, using the natural amino acid L-ornitine, which is the only one available in Nature. In this case, we decided to try the link between the amino acid and the polyenic chain using the amino group located at C-2′ (see Scheme 2). The synthesis of **3** (Scheme 4) started from protected ester **8**, which had been previously used in the synthesis of **2**. After ester hydrolysis under strong basic conditions, we performed the direct amidation of **11** with commercial available hydrochloride **12** using a methodology recently developed by our group,[15] yielding the corresponding amide **13** (50% yield). After three consecutive cleavages of different protecting groups, the derivative *L*-**3** (67% yield) was isolated. Comparison of ^1H and ^{13}C NMR observed for *L*-**3** with those previously described for [12]-granadaene (**1**)[7] (see Tables 3 and 4) showed the close relationship between these two compounds. The signals corresponding to the amino acid subunit and the polyenic chain match almost perfectly (Tables 3 and 4). Therefore, we could confirm that [12]-granadaene (**1**) has a L-ornitinite amino acid in its structure, and the link of the amino acid to the rest of the molecule occurs using the amino group located at C-2′, ruling out the possible link using the amine at C-5′.



Scheme 3. Synthesis of sugar-containing derivative (*R*,α)-**2** from (*R*)-**5**.

Table 1
Key ^{1}H NMR signals for **1** and **2**

H	**1**[a] ^{1}H NMR ppm (multiplicity, *J*)	**2**[a] ^{1}H NMR ppm (multiplicity, *J*)
1″	4.63 (d, 1.5)	4.68 (bs)
2″	3.52 (dd, 3, 1.5)	3.55 (bs)
3″	3.39 (dd, 9, 3)	3.41 (m)
4″	3.16 (t, 9)	3.19 (t, 9)
5″	3.43 (dd, 9, 6)	3.45 (m)
6″	1.09 (d, 6)	1.12 (d, 6.1)
2	6.1 (d, 15.1)	5.91 (d, 15.4)
3	7.1 (dd, 15.1, 11)	7.22 (dd, 15.3, 10.6)
4, 6–24	6.5–6.2 (m)	6.26 (m)[b]
25	5.72 (dt, 14.5, 7.2)	6.34 (dd, 14.8, 11.2)
26	2.25 (m)	2.35 (m)
27	3.69 (sext, 6.1)	3.78 (dq, 12.4, 6.2)
28	1.03 (d, 6.1)	1.09 (d, 6.1)

[a] Spectra were recorded in DMSO-d_6, 0.1% TFA-d.
[b] Refer to H-4.

Table 2
^{13}C NMR signals for **1** and **2**

C	**1**[a] ^{13}C NMR ppm	**2**[a] ^{13}C NMR ppm
1″	97.4	97.6
2″	70.8	70.6
3″	70.5	70.5
4″	71.8	71.9
5″	68.8	68.7
6″	17.7	17.8
2	124.0	119.4
3	138.8	144.6
4, 6–24	130–136	141.0[b]
25	131.7	130.0
26	39.9	39.7
27	71.0	71.0
28	18.7	18.8

[a] Spectra were recorded in DMSO-d_6, 0.1% TFA-d.
[b] Refer to C-4.

Table 3
Key ^{1}H NMR signals for **1** and *L*-**3**

H	**1** [a] ^{1}H NMR ppm (multiplicity, *J*)	*L*-**3** [a] ^{1}H NMR ppm (multiplicity, *J*)
2	6.1 (d, 15.1)	5.91 (d, 15.3)
3	7.1 (dd, 15.1, 11)	7.16 (dd, 14.4, 10.8)
4, 6–24	6.5–6.2 (m)	6.23 (m)[b]
25	5.72 (dt, 14.5, 7.2)	6.05 (dt, 14.5, 7.2)
26	2.25 (m)	2.36 (m)
27	3.69 (sext, 6.1)	3.98 (m)
28	1.03 (d, 6.1)	1.20 (d, 6.1)
2′	4.32 (dd, 8.9, 5)	4.33 (dd, 8.3, 6.7)
3′	1.80 (m), 1.64 (m)	1.84 (m), 1.65 (m)
4′	1.59 (m)	1.59 (m)
5′	2.77 (t, 6.7)	2.80 (bs)

[a] Spectra were recorded in DMSO-d_6, 0.1% TFA-d.
[b] Refer to H-4.

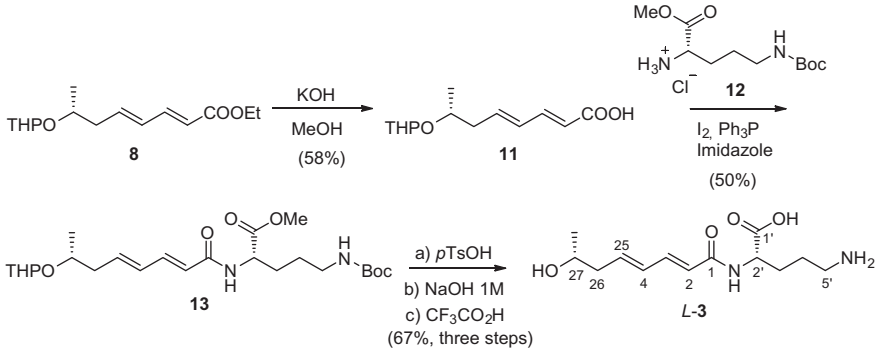
Table 4
^{13}C NMR signals for **1** and *L*-**3**

C	**1**[a] ^{13}C NMR ppm	*L*-**3**[a] ^{13}C NMR ppm
2	124.0	124.3
3	138.8	138.6
4, 6–24	130–136	133.2[b]
25	131.7	130.4
26	39.9	40.0
27	71.0	71.0
28	18.7	18.7
2′	51.1	51.4
3′	27.9	28.0
4′	23.5	23.7
5′	38.1	38.1

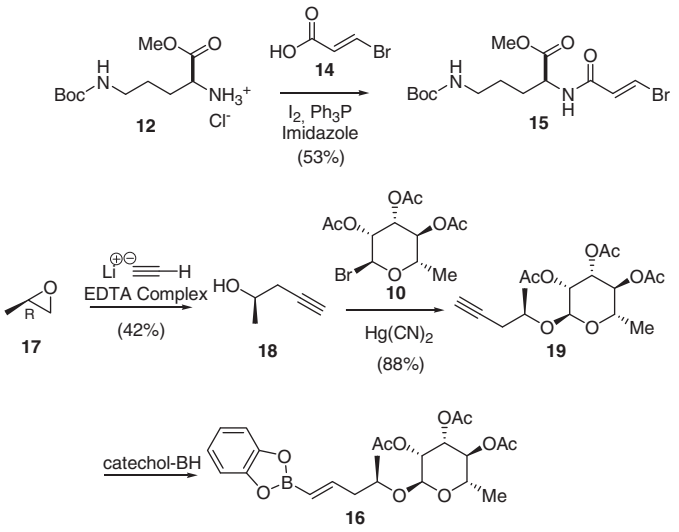
[a] Spectra were recorded in DMSO-d_6, 0.1% TFA-d.
[b] Refer to C-4.

Although the synthesis depicted before were useful to determinate each of the unknown structural features of granadaene (**1**), a new synthetic approach was necessary to obtain a compound containing all the important structural aspects of **1**. In this sense, the synthesis of granadaene-type compounds could be accomplished taking into account a modular and convergent approach, ensuring the stereochemistry of the polyprenic chain (Scheme 5). Within this context, Suzuki–Miyaura coupling has been extensively used in the synthesis of stereocontrolled polyenic chains.[16,17] The chemical compatibility of this methodology also allows the presence of L-rhamnose (**16**) and L-ornithine (**15**) fragments in the corresponding subunits. Moreover, this approach would also allow the introduction of different sugars and/or amino acids in the final molecule, which could allow to carry out further biological studies in more details.[18] To check the feasibility of our approach, we focussed in the synthesis of the shortest member of the family, [2]-granadaene (**4**),[6] which posses all the structural motifs present in natural [12]-granadaene (**1**) (Schemes 5 and 6).

In this case, a direct Suzuki–Miyaura coupling between fragments **15** and **16** would yield the desired compound **4** (Scheme 6). Amino acid derivative **15** was prepared from commercially available protected L-ornithine **12** and 3-bromoacrylic acid **14**,[19] as previously described in the synthesis of *L*-**3**.[15] On the other hand, fragment **16** was readily prepared from epoxide **17** in a few steps,



Scheme 4. Synthesis of amino acid-containing derivative *L*-**3**.



Scheme 5. Preparation of fragments **15** and **16**.

with complete stereochemical control (Scheme 5). As we established before, it is known that the glycosylation reaction of rhamnose derivative **10** mediated by $Hg(CN)_2$ yields the corresponding α anomer, and the absolute configuration at C-27 was determined as *R*. Thus, the preparation of **16** had to include all these structural requirements. In this case, the stereochemical control on C-27 was obtained by nucleophylic opening of commercial available enantiopure epoxide (*R*)-**17** by lithium acetylide-EDTA complex, to yield alcohol **18**[20] in modest yield (42%). Then, alcohol **18** was efficiently glycosylated with **10**, under the conditions indicated in Scheme 3. Finally, hydroboration of alkyne moiety with catecholborane in THF gave intermediate **16**, which was subsequently used in the next step. Suzuki–Miyaura coupling of both fragments **15** and **16** generated diene (*E,E*)-**20**, which possesses the main framework of **1**. Finally, deprotection in two steps (saponification of acetate groups, and removing of Boc group and ester hydrolysis under acidic conditions) yielded analogue **4** in 65% yield (two steps). Again the chemical shifts for compound **4** are in agreement with those reported for compound **1** confirming again its fundamental structural features (see Tables 5 and 6).

3. Conclusions

In conclusion, a modular and flexible approach to compounds of granadaene family has been described. The absolute configuration at C-27 and the stereochemistry of the anomeric center were also determined, as well as the position of the link of the amino acid fragment to the rest of the compound. We are now working in the total synthesis of [12]-granadaene (**1**) and some other derivatives including other different sugars and amino acids to check their biological activities.

Table 5
Key ^{1}H NMR signals for **1** and **4**

H	**1**[a] ^{1}H NMR ppm (multiplicity, *J*)	**4**[a] ^{1}H NMR ppm (multiplicity, *J*)
1″	4.63 (d, 1.5)	4.66 (bs)
2″	3.52 (dd, 3, 1.5)	3.53 (bs)
3″	3.39 (dd, 9, 3)	3.39 (dd, 9.4, 3.1)
4″	3.16 (t, 9)	3.18 (t, 9)
5″	3.43 (dd, 9, 6)	3.42 (dd, 9.2, 6.3)
6″	1.09 (d, 6)	1.10 (d, 6.1)
2	6.1 (d, 15.1)	6.04 (d, 15.3)
3	7.1 (dd, 15.1, 11)	7.01 (dd, 15, 11)
4, 6–24	6.5–6.2 (m)	6.25 (m)[b]
25	5.72 (dt, 14.5, 7.2)	6.09 (m)
26	2.25 (m)	2.32 (m)
27	3.69 (sext, 6.1)	3.74 (dt, 12.2, 6.1)
28	1.03 (d, 6.1)	1.06 (d, 6)
2′	4.32 (dd, 8.9, 5)	4.30 (dt, 8.3, 6.7)
3′	1.80 (m), 1.64 (m)	1.80 (m,), 1.63 (m)
4′	1.59 (m)	1.57 (m,)
5′	2.77 (t, 6.7)	2.79 (bs)

[a] Spectra were recorded in DMSO-d_6, 0.1% TFA-d.
[b] Refer to H-4.

Table 6
^{13}C NMR signals for **1** and **4**

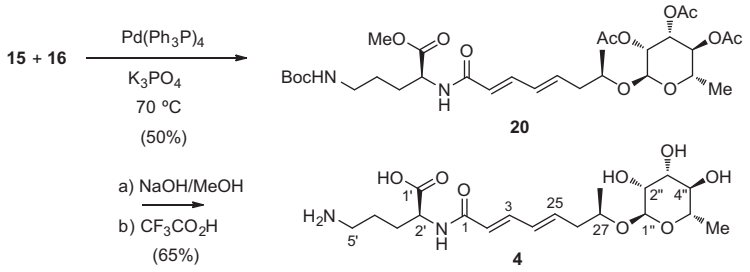
C	**1** [a] ^{13}C NMR ppm	**4** [a] ^{13}C NMR ppm
1″	97.4	97.6
2″	70.8	70.7
3″	70.5	70.6
4″	71.8	71.9
5″	68.8	68.8
6″	17.7	17.9
2	124.0	123.1
3	138.8	138.4
4, 6–24	130–136	130.3[b]
25	131.7	139.7
26	39.9	40.0
27	71.0	71.0
28	18.7	18.8
2′	51.1	51.3
3′	27.9	28.2
4′	23.5	23.8
5′	38.1	38.5

[a] Spectra were recorded in DMSO-d_6, 0.1% TFA-d.
[b] Refer to C-4.

4. Experimental Section

4.1. General details

Deoxygenated solvents and reagents were used for all reactions. THF was freshly distilled from Na. CH_2Cl_2 was freshly distilled from P_2O_5. Products were purified by flash chromatography on Merck



Scheme 6. Synthesis of [2]-granadaene (**4**).

silica gel 50. Yields refer to analytically pure samples. NMR spectra were recorded in NMR 400 MHz and 500 MHz spectrometers. The following known compounds were isolated as pure samples and showed NMR spectra matching those of the reported compounds: **6**,[10] **10**,[12] **14**,[19] and **18**.[20]

4.2. Synthesis of (*R*,α)-2

4.2.1. Preparation of compound 8

To a solution of phosphonate **7** (916 mg, 3.66 mmol) in dry THF (10 mL) at 0 °C, DMPU (913 mg, 7.12 mmol) and *n*-BuLi (4.07 mmol, 1.63 mL, 2.5 M in hexane) were added and the new mixture was stirred for 30 min. Then, the mixture was cooled to −78 °C and a solution of aldehyde **6**[10] (350 mg, 2.03 mmol) in dry THF (10 mL) was slowly added. The mixture was stirred for 1 h at −78 °C, warmed until 0 °C and stirred for additional 3 h. Then, EtOAc was added and the organic layer was washed with saturated solution of NH_4Cl. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 8/2) to yield ester **8** (393 mg, 72%). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.19 (m, 1H), 6.28–6.04 (m, 2H), 5.80 (d, J = 15.4 Hz, 1H), 4.72–4.69 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.95–3.81 (m, 2H), 3.50–3.42 (m, 2H), 2.48–2.26 (m, 2H), 1.84–1.76 (m, 1H), 1.72–1.64 (m, 2H), 1.46–1.58 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 1.12 (d, J = 6.2, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3 (C), 144.7 (CH), 140.1 (CH), 130.7 (CH), 119.9 (CH), 98.3 (CH), 72.6 (CH), 62.8 (CH_2), 60.3 (CH_2), 40.0 (CH_2), 31.1 (CH_2), 25.6 (CH_2), 21.6 (CH_3), 19.9 (CH_2), 14.4 (CH_3); HRMS-ESI(+) for $C_{15}H_{24}O_4Na$ Calcd 291.1566. Found 291.1573 $[M+Na]^+$.

4.2.2. Synthesis of alcohol 9

To a solution of ester **8** (60 mg, 0.22 mmol) in EtOH (10 mL) at rt, *p*TsOH (3 mg, 0.011 mmol) were added and the mixture was stirred for 16 h. Then, EtOAc was added and the organic layer was washed with brine. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 7/3) to yield alcohol **9** (40 mg, 97%). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.21 (m, 1H), 6.26 (dd, J = 15.2, 10.8 Hz, 1H), 6.17–6.08 (m, 2H), 5.83 (d, J = 15.4 Hz, 1H), 4.20 (q, J = 7.2 Hz, 2H), 3.92 (dd, J = 12.0, 6.3 Hz, 1H), 2.34 (dd, J = 12.8, 6.4 Hz, 2H), 1.31–1.27 (m, 3H), 1.23 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2 (C), 144.5 (CH), 139.8 (CH), 131.2 (CH), 120.4 (CH), 67.2 (CH), 60.4 (CH_2), 42.8 (CH_2), 23.2 (CH_3), 14.4 (CH_3); HRMS-ESI(+) for $C_{10}H_{17}O_3$ Calcd 185.1172. Found 185.1188 $[M+H]^+$.

4.2.3. Preparation of (*R*,α)-2

To a solution of alcohol **9** (40 mg, 0.22 mmol) and $Hg(CN)_2$ (50 mg, 0.2 mmol) in dry CH_3CN (10 mL), bromorhamnose **10** (115 mg, 0.33 mmol) was added in three portions during 3 h, and the mixture was stirred for additional 4 h. Then, the solvent was removed. The residue was solved in CH_2Cl_2 and washed with 1 M solution of KBr, saturated solution of $NaHCO_3$ and water. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 7/3) to yield the corresponding glycosylation product (90 mg, 91%). This compound was solved in MeOH (10 mL) and K_2CO_3 (136 mg, 1 mmol) was added, and the mixture was stirred for 10 min. Then, brine was added and the mixture was extracted with EtOAc, dried (anhyd Na_2SO_4) and the solvent remove. Compound (*R*,α)-**2** was obtained (65 mg, 98%) without further purification. Colorless oil; $[\alpha]_D^{25}$ + 18.5 (c = 0.85, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.22 (dd, J = 15.3, 10.6 Hz, 1H), 6.34 (dd, J = 14.8, 11.2 Hz, 1H), 6.26 (ddd, J = 12.1, 7.4, 4.0 Hz, 1H), 5.91 (d, J = 15.4 Hz, 1H), 4.68 (bs, 1H), 3.78 (dq, J = 12.4, 6.2 Hz, 1H), 3.68 (s, 3H), 3.65–3.49 (m, 1H), 3.47–3.43 (m, 1H), 3.43–3.38 (m, 1H), 3.19 (t, J = 9.2 Hz, 1H), 2.38–2.32 (m, 2H), 1.12 (d, J = 6.1 Hz, 3H), 1.09 (d, J = 6.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.2 (C), 144.6 (CH), 141.0 (CH), 130.0 (CH), 119.4 (CH), 97.6 (CH), 71.9 (CH), 71.0 (CH), 70.6 (CH), 70.5 (CH), 68.7 (CH), 59.7 (CH_2), 51.2 (CH_3), 18.8 (CH_3), 17.8 (CH_3); HRMS-ESI(+) for $C_{15}H_{24}O_7Na$ Calcd 339.1414, Found 339.1422 $[M+Na]^+$.

4.3. Synthesis of (*L*)-3

4.3.1. Saponification of ester 8

Ester **8** (155 mg, 0.58 mmol) was treated with 5% solution of KOH in MeOH (15 mL) for 30 h. Then, 2 N HCl was added until pH 6, the mixture was diluted with EtOAc and the organic layer was washed with water. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed to yield acid **11** (126 mg, 91%) without further purification. Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.28 (m, 1H), 6.31–6.09 (m, 2H), 5.86–5.77 (m, 1H), 4.78–4.62 (m, 1H), 3.97–3.80 (m, 2H), 3.54–3.43 (m, 1H), 2.52–2.28 (m, 2H); 1.96–1.44 (m, 6H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (C), 146.7 (CH), 141.1 (CH), 130.3 (CH), 118.9 (CH), 96.1 (CH), 70.4 (CH), 62.5 (CH_2), 41.0 (CH_2), 31.1 (CH_2), 25.5 (CH_2), 19.8 (CH_2), 18.6 (CH_3).

4.3.2. Preparation of amide 13

To a solution of I_2 (94 mg, 0.37 mmol) in dry CH_2Cl_2 (10 mL), Ph_3P (97 mg, 0.37 mmol) was added, giving the solution a brown–yellow color. Then, imidazole (89 mg, 1.28 mmol) was added, changing the color to light yellow. Subsequently, acid **11** (69 mg, 0.29 mmol) was added and the solution was stirred for 5 min at room temperature, and then commercial amine **12** (86 mg, 0.35 mmol) was added. The mixture was stirred until completely consumption of the starting material (checked by TLC, around 24 h). Then, CH_2Cl_2 was added, and the solution was washed with 2 N HCl and water before being dried with anhyd Na_2SO_4, and the solvent removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 1/1) to yield amide **13** (68 mg, 50%). Colorless oil; $[\alpha]_D^{25}$ + 17.3 (c = 0.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.16 (m, 1H), 6.52–5.96 (m, 2H), 5.94–5.81 (m, 1H), 4.75–4.57 (m, 1H), 4.22–4.15 (m, 1H), 3.81–3.75 (m, 2H), 3.74 (s, 3H), 3.55–3.44 (m, 1H), 3.20–3.07 (m, 1H), 2.75–2.58 (m, 4H), 1.97–1.75 (m, 6H), 1.77–1.47 (m, 4H), 1.43 (s, 9H), 1.22 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (C), 166.1 (C), 156.2 (C), 142.1 (CH), 133.3 (CH), 131.4 (CH), 127.8 (CH), 113.1 (CH),79.2, 70.4 (CH), 62.5 (CH_2), 52.5 (CH_3), 45.8 (CH), 39.9 (CH_2), 33.1 (CH_2), 29.8 (CH_2), 28.4 (CH_3), 26.4 (CH_2), 22.9 (CH_2), 19.9 (CH_2), 18.6 (CH_3); HRMS-ESI(+) for $C_{24}H_{40}O_7N_2Na$ Calcd 491.2727. Found 491.2737 [M+Na]+.

4.3.3. Synthesis of model compound (*L*)-3

To a solution of amide **13** (65 mg, 0.14 mmol) in EtOH (10 mL) at rt, *p*TsOH (1.3 mg, 0.007 mmol) was added and the mixture was stirred for 24 h. Then, EtOAc was added and the organic layer was washed with brine. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed. The residue was submitted to flash chromatography on silicagel (EtOAc) to yield the corresponding alcohol (54 mg, 97%). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, J = 14.8, 10.8 Hz, 1H), 6.22 (dd, J = 15.2, 10.8 Hz, 1H), 6.08 (dd, J = 14.4, 7.2 Hz, 1H), 5.86 (d, J = 15.2 Hz, 1H), 4.60 (m, 2H), 3.80 (q, J = 6.4 Hz, 2H), 3.68 (s, 3H), 3.10 (m, 1H), 2.45–2.20 (m, 2H), 1.60–1.84 (m, 8H), 1.38 (s, 9H), 1.21 (d, J = 6.2 Hz, 3H). This alcohol was dissolved in MeOH (10 mL) and NaOH 1 M (0.28 mL, 0.28 mmol) were added and the mixture was stirred for 16 h. Then, amberlyst was added until to reach pH 7, the mixture was fil-

tered and the solvent removed. The residue was submitted to flash chromatography on silicagel (EtOAc/MeOH 8/2) to yield the corresponding acid (38 mg, 74%). This compound was immediately used in the next step. Colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 7.11 (dd, J = 15.0, 10.8 Hz, 1H), 6.29–6.20 (m, 1H), 6.10 (dd, J = 14.8, 7.3 Hz, 1H), 6.02 (d, J = 15.2 Hz, 1H), 3.87–3.78 (m, 1H), 3.72 (s, 1H), 3.03 (t, J = 6.3 Hz, 2H), 2.36 (dd, J = 12.9, 6.5 Hz, 2H), 1.86 (dd, J = 12.3, 4.9 Hz, 2H), 1.72–1.61 (m, 2H), 1.40 (s, 9H), 1.13 (d, J = 6.0 Hz, 3H). This acid (38 mg, 0.1 mmol) was subsequently treated with trifluoroacetic acid (0.2 mL, 2.6 mmol) for 15 min. Then, the excess of acid was removed, to yield amine (*L*)-**3** (25 mg, 93%). Colorless oil; ^{1}H NMR (400 MHz, DMSO-d_6 + 0.1% CF_3CO_2D) δ 7.16 (dd, J = 14.4, 10.8 Hz, 1H), 6.23 (dd, J = 15.0, 10.6 Hz, 1H), 6.05 (dd, J = 14.5, 7.2 Hz, 1H), 5.91 (d, J = 15.3 Hz, 1H), 4.33 (dt, J = 8.3, 6.7 Hz, 1H), 3.98 (td, J = 13.2, 5.9 Hz, 1H), 2.80 (bs, 2H), 2.20–2.45 (m, 2H), 1.89–1.83 (m, 1H), 1.68–1.63 (m, 1H), 1.62–1.56 (m, 2H), 1.20 (d, J = 6.1 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6 + 0.1% CF_3CO_2D) δ 175.4 (C), 164.9 (C), 138.6 (CH), 133.2 (CH), 130.4 (CH), 124.3 (CH), 71.1 (CH), 51.4 (CH), 40.0 (CH_2), 38.1 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 18.7 (CH_3).

4.4. Synthesis of model compound [2]-granadaene (4)

4.4.1. Preparation of amide 15

To a solution of I_2 (404 mg, 1.59 mmol) in dry CH_2Cl_2 (10 mL), Ph_3P (417 mg, 1.59 mmol) was added, giving the solution a brown–yellow color. Then, imidazole (324 mg, 4.66 mmol) was added, changing the color to light yellow. Subsequently, acid **14**[19] (176 mg, 1.17 mmol) was added and the solution was stirred for 5 min at room temperature, and then commercial amine **12** (300 mg, 1.06 mmol) was added. The mixture was stirred until completely consumption of the starting material (checked by TLC, around 24 h). Then, CH_2Cl_2 was added, and the solution was washed with 2 N HCl and water before being dried with anhyd Na_2SO_4, and the solvent removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 6/4) to yield amide **15** (214 mg, 53%). Colorless oil; $[\alpha]_D^{25}$ + 12.6 (c = 0.52, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 13.5 Hz, 1H), 6.58 (d, J = 13.4 Hz, 1H), 4.64 (dd, J = 12.3, 7.3 Hz, 1H), 3.75 (s, 3H), 3.21–3.06 (m, 2H), 1.96–1.84 (m, 2H), 1.79–1.66 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7 (C), 163.6 (C), 156.4 (C), 130.6 (CH), 123.3 (CH), 95.5 (CH), 79.4 (C), 52.6 (CH_3), 39.9 (CH_2), 29.0 (CH_2), 28.4 (CH_3), 26.4 (CH_2); HRMS-ESI(+) for $C_{14}H_{23}O_5N_2Br$ Calcd 401.0682. Found 401.0699 $[M+Na]^+$.

4.4.2. Synthesis of alkyne 19

To a solution of alcohol **18**[20] (330 mg, 3.57 mmol) and $Hg(CN)_2$ (812 mg, 2.21 mmol) in dry CH_3CN (6 mL), bromorhamnose **10** (1.89 g, 5.36 mmol) was added in three portions during 3 h, and the mixture was stirred for additional 4 h. Then, the solvent was removed. The residue was solved in CH_2Cl_2 and washed with 1 M solution of KBr, saturated solution of $NaHCO_3$ and water. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 7/3) to yield alkyne **19** (1.23 g, 97%). Colorless oil; $[\alpha]_D^{25}$ − 76.5 (c = 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (d, J = 3.4 Hz, 1H), 5.30 (d, J = 3.3 Hz, 1H), 5.19 (d, J = 1.6 Hz, 1H), 5.07 (t, J = 10.0 Hz, 1H), 4.10 (dt, J = 11.8, 8.2 Hz, 1H), 3.91 (dq, J = 12.3, 6.3 Hz, 1H), 2.44 (qdd, J = 16.8, 6.1, 2.6 Hz, 2H), 2.18 (s, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.26 (d, J = 6.1 Hz, 3H), 1.21 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (C), 170.2 (C), 170.1 (C), 95.7 (CH), 81.0 (C), 72.4 (CH), 71.3 (CH), 70.5 (CH), 70.2 (CH), 69.3 (CH), 66.7 (CH), 26.9 (CH_2), 21.1 (CH_3), 21.0 (CH_3), 20.9 (CH_3), 19.0 (CH_3), 17.4 (CH_3); HRMS-ESI(+) for $C_{17}H_{24}O_8Na$ Calcd 379.1363. Found 379.1370 $[M+Na]^+$.

4.4.3. Preparation of intermediate 20

To a flask containing alkyne **19** (307 mg, 0.88 mmol), catecholborane (105 mg, 0.88 mmol) was added under Ar atmosphere and the mixture was stirred at 70 °C for 3 h. Then, the mixture was solved in dry and strictly deoxygenated THF (5 mL) and a mixture of **15** ((120 mg, 0.33 mmol), $Pd[PPh_3]_4$ (19 mg, 0.017 mmol) and K_3PO_4 (162 mg, 0.99 mmol) in THF (5 mL) was added. This new mixture was stirred at 70 °C for 48 h. Then, brine was added and the mixture was extracted with EtOAc. The organic layer was dried (anhyd Na_2SO_4) and the solvent was removed. The residue was submitted to flash chromatography on silicagel (hexane/EtOAc 7/3) to yield **20** (110 mg, 55%). Colorless oil; $[\alpha]_D^{25}$ − 30.4 (c = 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (dd, J = 14.9, 10.9 Hz, 1H), 6.22 (dd, J = 14.9, 11.0 Hz, 1H), 6.09–5.99 (m, 1H), 5.87 (d, J = 15.0 Hz, 1H), 5.24 (dd, J = 10.1, 3.3 Hz, 1H), 5.16 (dd, J = 3.4, 1.8 Hz, 1H), 5.03 (t, J = 9.9 Hz, 1H), 4.82 (s, 1H), 4.65 (dd, J = 12.4, 5.2 Hz, 1H), 3.89–3.78 (m, 1H), 3.73 (s, 3H), 3.12 (d, J = 6.1 Hz, 2H), 2.47–2.29 (m, 2H), 2.13 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.87 (dd, J = 14.2, 6.1 Hz, 2H), 1.75–1.65 (m, 3H), 1.42 (s, 9H), 1.17 (d, J = 6.3 Hz, 3H), 1.14 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0 (C), 170.3 (C), 170.2 (C), 170.1 (C), 166.0 (C), 156.2 (C), 141.5 (CH), 138.4 (CH), 131.1 (CH), 122.3 (CH), 95.1 (CH), 72.6 (CH), 71.2 (CH), 70.6 (CH), 69.3 (CH), 66.8 (CH), 52.6 (CH), 52.1 (CH), 40.5 (CH_2), 40.1 (CH_2), 32.3 (C), 29.8 (CH_2), 28.5 (CH_3), 26.4 (CH_2), 21.1 (CH_3), 21.0 (CH_3), 20.9 (CH_3), 19.0 (CH_3), 17.5 (CH_3); HRMS-ESI(+) for $C_{31}H_{48}O_{13}N_2Na$ Calcd 679.3048. Found 679.3075 $[M+Na]^+$.

4.4.4. Synthesis of [2]-granadaene (4)

To a solution of amide **20** (110 mg, 0.18 mmol) in MeOH (10 mL), NaOH 1 M (1.26 mL, 1.26 mmol) was added and the mixture was stirred for 16 h. Then, amberlyst was added until pH 7, the mixture was filtered and the solvent was removed. The residue was submitted to flash chromatography on silicagel (EtOAc/MeOH 9/1) to yield the corresponding alcohol (51 mg, 55%). Colorless oil; $[\alpha]_D^{25}$ − 3.9 (c = 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.19 (dd, J = 15.0, 10.8 Hz, 1H), 6.39–6.28 (m, 1H), 6.18 (dd, J = 14.8, 7.3 Hz, 1H), 6.11 (d, J = 15.2 Hz, 1H), 4.40 (s, 1H), 3.96–3.86 (m, 1H), 3.80 (s, 3H), 3.70 (dd, J = 9.3, 3.3 Hz, 1H), 3.46–3.40 (m, 1H), 3.11 (t, J = 6.3 Hz, 2H), 2.52–2.37 (m, 2H), 1.99–1.88 (m, 2H), 1.81–1.69 (m, 2H), 1.63–1.54 (m, 3H), 1.48 (s, 9H), 1.29 (d, J = 6.1 Hz, 3H), 1.21 (d, J = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1 (C), 167.8 (C), 158.5 (C), 144.0 (CH), 141.7 (CH), 139.5 (CH), 132.1 (CH), 99.3 (CH), 79.8 (CH), 73.9 (CH), 73.0 (CH), 72.8 (CH), 70.1 (CH), 48.4 (CH), 41.6 (CH_2), 41.1 (CH_2), 37.9 (CH_2), 32.3 (C), 28.8 (CH_3), 25.9 (CH_2), 19.2 (CH_3), 18.0 (CH_3); HRMS-ESI(+) for $C_{25}H_{42}O_{10}N_2Na$ Calcd 553.2737. Found 553.2746 $[M+Na]^+$. This alcohol (51 mg, 0.1 mmol) was treated with trifluoroacetic acid (0.3 mL, 3.8 mmol) for 5 min. Then, the excess of acid was removed, to yield **4** (37 mg, 91%). Colorless oil; ^{1}H NMR (600 MHz, DMSO-d_6 + 0.1% CF_3CO_2D) δ 7.01 (dd, J = 15.0, 11.0 Hz, 1H), 6.25 (dd, J = 14.9, 11.2 Hz, 1H), 6.09 (m, 1H), 6.04 (d, J = 15.3 Hz, 1H), 4.66 (s, 1H), 4.30 (dt, J = 8.3, 6.7 Hz, 1H), 3.74 (td, J = 12.2, 6.1 Hz, 1H), 3.53 (s, 1H), 3.42 (dd, J = 9.2, 6.3 Hz, 1H), 3.39 (dd, J = 9.4, 3.1 Hz, 1H), 3.18 (t, J = 9.3 Hz, 1H), 2.79 (s, 2H), 2.32 (m, 2H), 1.80 (dt, J = 11.5, 7.3 Hz, 1H), 1.63 (dd, J = 14.1, 6.1 Hz, 1H), 1.61–1.54 (m, 2H), 1.10 (d, J = 6.1 Hz, 3H), 1.06 (d, J = 6.0 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6 + 0.1% CF_3CO_2D) δ 173.3 (C), 165.3 (C), 139.7 (CH), 138.4 (CH), 130.3 (CH), 123.1 (CH), 97.6 (CH), 71.9 (CH), 71.0 (CH), 70.7 (CH), 70.6 (CH), 68.8 (CH), 51.3 (CH), 40.1 (CH_2), 38.5 (CH_2), 28.2 (CH_2), 23.8 (CH_2), 18.8 (CH_3), 17.9 (CH_3).

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