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Synthetic studies of neoclerodane diterpenoids from *Salvia splendens* and evaluation of opioid receptor affinity

Gianfranco Fontana [a], Giuseppe Savona [a,*], Benjamín Rodríguez [b,*], Christina M. Dersch [c], Richard B. Rothman [c], Thomas E. Prisinzano [d]

[a] *Dipartimento di Chimica Organica 'E. Paternò', Università degli Studi di Palermo, Parco d'Orleans 2, I-90128 Palermo, Italy*
[b] *Instituto de Química Orgánica, Consejo Superior de Investigaciones Científicas (CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain*
[c] *Clinical Psychopharmacology Section, IRP, NIDA, NIH, DHHS, Baltimore, MD 21224, USA*
[d] *Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS 66045, USA*

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ABSTRACT

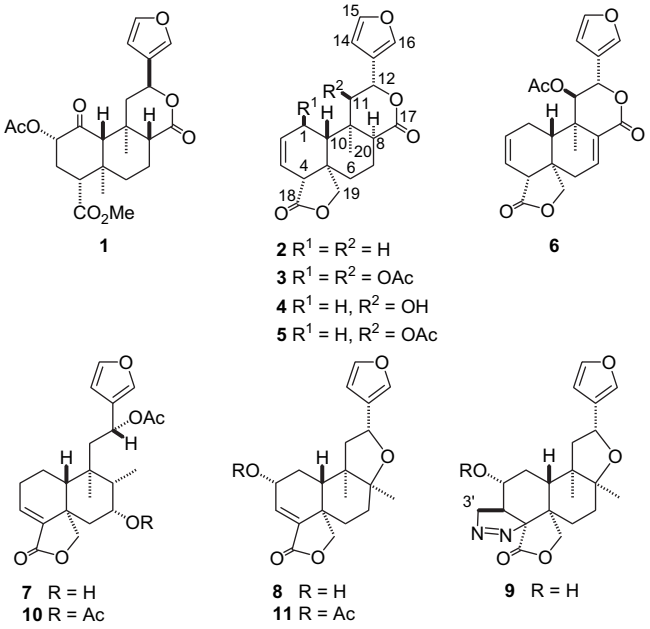
Salvinorin A (**1**), a neoclerodane diterpene from the hallucinogenic mint *Salvia divinorum*, is the only known non-nitrogenous and specific κ-opioid agonist. Several structural congeners of **1** isolated from *Salvia splendens* (**2**–**8**) together with a series of semisynthetic derivatives (**9**–**24**), some of which possess a pyrazoline structural moiety (**9**, **19**–**22**), have been tested for affinity at human μ, δ, and κ opioid receptors. None of these compounds showed high affinity binding to these receptors. However, **10** showed modest affinity for κ receptors suggesting that other natural neoclerodanes from different *Salvia* species may possess opioid affinity.

1. Introduction

Salvinorin A (**1**), one of the neoclerodane diterpenes of *Salvia divinorum* Epling & Játiva (Labiatae), is a highly unusual drug that exerts its hallucinogenic effects as a potent and selective agonist of the κ-opioid receptor.[1] In the past few years, intensive research on the pharmacology and chemical transformations of salvinorin A have been made,[1–6] and its biosynthetic pathway has been elucidated[7] and its asymmetric total synthesis has been achieved.[8]

Recently,[9] some of us have reinvestigated the diterpene constituents of *Salvia splendens* Sellow ex Roem. & Schult isolating four new neoclerodanes (salvisplendins A–D, **4**, **6**, **7**, and **8**, respectively) together with the artifact **9**, that results from **8** by treatment of the crude extract with diazomethane,[9] and salviarin (**2**),[10] splendidin (**3**),[11] and splenolides A and B (**5**),[12] all of them previously reported as constituents of this species.[9–13]

Taking into account the structural similarity between salvinorin A (**1**) and the diterpenes found in *S. splendens* (**2**–**9**), we decided to test these substances and two of their available derivatives (**10** and **11**)[9] in order to explore whether such compounds may be useful as neuropharmacological agents.



Moreover, we have now obtained several new semisynthetic derivatives (**12**–**24**) starting from the more abundant constituents

* Corresponding authors. Tel.: +39 091 597193; fax: +39 091 596825 (G.S.); tel.: +34 91 5622900; fax: +34 91 5644853 (B.R.).
E-mail addresses: giussav@unipa.it (G. Savona), iqor107@iqog.csic.es (B. Rodríguez).

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of *S. splendens* (**2**, **3**, **5**, **7**, and **9**),[9,13] and these compounds were also assayed as potential agonists at the opioid receptors. Three clerodanes of *S. splendens*, salviarin (**2**), splendidin (**3**), and splenolide B (**5**), have recently been tested for affinity at opioid receptors and none of these diterpenes showed significant binding to any of the opioid receptor subtypes.[14] This work, however, demonstrates the potential utility of evaluating other neoclerodanes for their interaction with opioid receptors.

2. Results and discussion

As reported previously,[1] salvinorin A (**1**) with the natural (β) configuration at C-8 shows higher affinity and efficacy at the κ-opioid receptor. Hence, our first objective was to epimerize the C-8 asymmetric center of salviarin, splendidin, and splenolide B (**2**, **3**, and **5**, respectively). Treatment of **2** with K_2CO_3 in MeOH yielded 8-*epi*-salviarin (**12**) (Scheme 1), a derivative, which had been obtained by Rodríguez-Hahn and co-workers using similar reaction conditions.[15] The complete assignment of the ^{1}H NMR spectrum together with other spectroscopic data of **12** have not been reported previously, and they are now included in Section 4. The β-configuration[16] of H-8 in **12** was supported by NOE experiments because irradiation at δ 2.62 (H-8) caused strong NOE enhancements in the H-6β, H-10β, and H-12β protons (+4.7, +5.1, and +15.3% NOE enhancement, respectively). This NOE behavior differs from that of the H-8α epimer **2**.[13] In addition, the coupling constant $J_{8\beta,7\alpha}$=11.9 Hz and $J_{8\beta,7\beta}$=3.4 Hz in **12**, as compared with those of **2** ($J_{8\alpha,7\alpha}$=4.0 Hz and $J_{8\alpha,7\beta}$=2.8 Hz),[13] further supported the β-configuration of H-8.

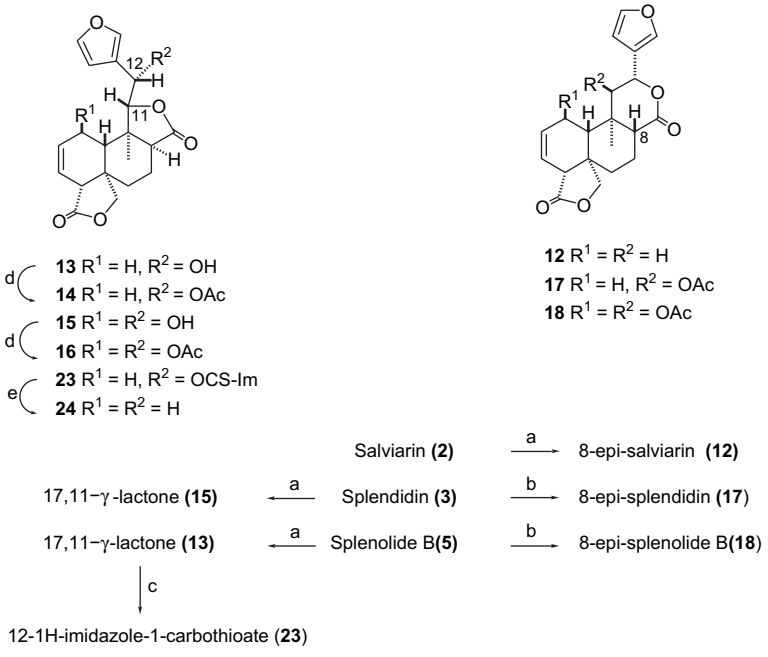
When **5** was treated with K_2CO_3 in MeOH under the same conditions that those described for **2** (see Section 4), only compound **13** ($C_{20}H_{22}O_6$) was obtained and it was transformed into a monoacetyl derivative (**14**, $C_{22}H_{24}O_7$) after treatment with Ac_2O–pyridine (Scheme 1). The ^{1}H and ^{13}C NMR spectra of **13** and **14** showed the presence of a 17,11-γ-lactone instead of the 17,12-δ-lactone in **5**. In particular, the HMBC correlations observed for **13** and **14** between the H-11 proton and the C-17 carbonyl carbon strongly support the presence of a 17,11-γ-lactone in these compounds. Moreover, the HMBC spectrum of **14** showed correlation between the carbonyl carbon of the acetate (δ 170.0) and a methine proton (δ 5.91, H-12), which in turn correlated with the C-13, C-14, and C-16 furanic carbons (δ 119.7, 109.9, and 141.9, respectively), thus confirming the proposed structures. NOE experiments on **13** and **14** established that the hydrogens at C-8 and C-11 were α- and β-oriented, respectively, since irradiation at Me-20 produced, among others, strong NOE enhancement in the signal of H-8α (+4.9% in **13** and +6.5% in **14**), whereas irradiation at δ 4.58 and 4.49 (H-11β in **13** and **14**, respectively) caused NOEs in the H-1β and H-10β protons (+8.1 and +8.4% in **13**; +8.1 and +11.5% in **14**, respectively) and not in Me-20.

Splendidin (**3**) also reacts in a similar way by treatment with Na_2CO_3 in MeOH giving **15** ($C_{20}H_{22}O_7$) (Scheme 1). The HMBC spectra of **15** and its diacetyl derivative **16** ($C_{24}H_{26}O_9$) showed the same correlations that those observed for **13** and **14**, and NOE experiments were also in agreement with an α- and β-orientation for their H-8 and H-11 protons, respectively.

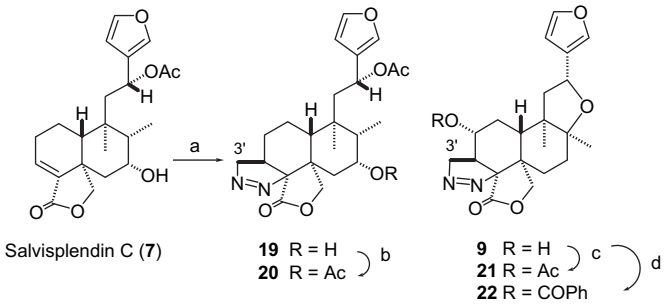
The epimerization of the C-8 position of salviarin (**2**) under basic conditions via enolate formation, followed by protonation from the opposite face[1,5] gave compound **12**. In the case of **3** and **5**, however, no epimerization was observed and this may be explained by an initial deacetylation at C-11, followed by a translactonization to the more stable cis fused 17,11-γ-lactones (**15** and **13**, respectively), in which epimerization at C-8 via an enolate is less favored.

Preparation of 8-*epi*-splenolide B (**17**) and 8-*epi*-splendidin (**18**) was achieved as follows (Scheme 1). Treatment of **5** with 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 solution for 72 h at room temperature yielded **17** (40%) together with minor quantities of **4** (15%) and **13** (10%), whereas identical treatment of **3** in anhydrous CH_2Cl_2 for 24 h at room temperature gave **18** (70% yield) and starting material (**3**, 15%). Like in **12**, the β-orientation of H-8 in **17** and **18** was strongly supported by NOE experiments and by the observed vicinal *J*(H,H) couplings of the H-8 methine and H-7 methylene protons (see Section 4).

Treatment of salvisplendin C (**7**) with an ethereal solution of diazomethane[9,17,18] gave the pyrazoline derivative **19** (Scheme 2).



Scheme 1. Reagents and conditions: (a) K_2CO_3/MeOH, rt, 4 h 65% (**12**), 73% (**13**), 58% (**15**); (b) DBU (10 equiv), CH_2Cl_2, rt, 72 h 40% (**17**), 24 h 70% (**18**); (c) CDI, DMAP (cat.), CH_2Cl_2, reflux, 2 h, 80%; (d) Ac_2O/Py 1:2, rt, quant.; (e) *n*-Bu_3SnH, AIBN (cat.), toluene, reflux, 6 h, 45%.



Scheme 2. Reagents and conditions: (a) CH_2N_2, Et_2O, 0 °C, 1 h, 73%; (b) Ac_2O/Py 1:2, 40 °C, one week, 55%; (c) Ac_2O/Py 1:2, rt, overnight, quant.; (d) PhCOCl (10 equiv), DMAP (cat.), CH_2Cl_2/Py 2:1, 0 °C to rt, overnight, 50%.

The regio- and stereochemistry of the pyrazoline moiety of **19** are identical to those of the already described derivative **9**,[9] as it was evidenced by comparison of their ^{1}H and ^{13}C NMR spectra (see Ref. 9 and Section 4).[19]

The acetyl derivatives **20**[19] and **21** and the benzoate **22** were prepared by standard procedures (Scheme 2) and their structural assignments were confirmed by extensive spectroscopic studies (see Section 4).

Finally, compound **24** was obtained from **13** by initial formation of the 12-1*H*-imidazole-1-carbothioate (**23**) followed by reaction of this intermediate with *n*-Bu_3SnH (Scheme 1).

Compounds **2**–**22** and **24** were then evaluated for affinity at opioid receptors. These analogues were screened to gain a greater understanding into the role of the orientation of the furan ring plays in the high affinity and selectivity of **1** for κ receptors. Several pyrazolines were also evaluated based on a previous report, which suggested that this ring system may potentially impart CNS depressant activity and/or anti-inflammatory activity.[20] Unfortunately, none of these compounds showed high affinity binding to human opioid receptors (K_i>10,000 nM). However, diterpene **10** showed weak affinity for κ opioid receptors (K_i=6910±570 nM). These results are not surprising given the differences in this series of agents compared to **1**. Some potential reasons for the lack of affinity compared to **1** are (a) the absence of a ketone at C-1; (b) the lack of a C-2 substituent; (c) the inverted stereochemistry at C-8; (d) the inverted stereochemistry at C-12; and (e) the incorporation of the C-4 carbomethoxy group into an additional ring. To begin to address possible explanations, several analogues were prepared. It was envisioned that inversion of the C-8 stereochemistry would result in enhanced affinity at opioid receptors. This structural change, however, did not increase the affinity of **3** (**18**) or **4** (**17**) for opioid receptors. These changes, however, are not parallel to the salvinorin A series. Given the previous SAR for **1** that the C-2 position has profound effects on affinity for opioid receptors,[21] **21** and **22** were synthesized from **9**. Interestingly, introduction of an acetyl group (**21**) did not increase affinity for κ opioid receptors and the addition of a benzoyl group (**22**) did not increase affinity for μ opioid receptors.

3. Conclusion

The data collected in this work indicate that the previous structure–activity relationships (SAR) may not be applicable to these neoclerodanes of general structure **9**. They also suggest that these two series are not binding in an identical manner and that the orientation of the furan ring is a key interaction in the mode of binding of neoclerodane diterpenes at opioid receptors.

4. Experimental

4.1. General

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. IR spectra were obtained on a Perkin–Elmer Spectrum One spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution, except for **13** and **15** (methanol-d_4), on a Varian INOVA 400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported in the δ scale and are referenced to residual $CHCl_3$ (δ 7.25) or methanol-d_4 (δ 3.30) signals for protons and to the solvent signals (δ_{CDCl_3} 77.00, δ_{CD_3OD} 49.00) for carbons. All the assignments for protons and carbons were in agreement with 2D COSY, gHSQC, gHMBC, and 1D NOESY spectra. Mass spectra were registered in the positive EI (70 eV) mode on a Hewlett–Packard 5973 instrument. Elemental analyses were conducted on a LECO CHNS-932 apparatus. Merck Si gel 7734 (70–230 mesh) deactivated with 15% (w/v) of water was used for gravity column chromatography and Si gel Merck LiChroprep 15–25/25–40 μm 1/1 was used for flash chromatography (elution under 0.7 psi of Ar). Merck 5554 Kieselgel 60 F_{254} sheets were used for thin-layer chromatographic analysis. Petroleum ether (bp 50–70 °C) was used for column chromatography.

4.2. Compounds for biological assays

Samples of compounds **2**–**11** and starting materials (**2**, **3**, **5**, **7**, and **9**) for obtaining the new derivatives **12**–**24** were available from a previous work.[9]

4.3. General procedure for reaction of compounds 2, 3, and 5 with potassium carbonate–methanol

The starting material (0.5 mmol) was treated with 5 mL of a saturated solution of K_2CO_3 in MeOH. The resulting solution was stirred for 4 h at room temperature and then neutralized by adding 2 N HCl and extracted with 5 portions (10 mL each) of 2:1 $CHCl_3$–EtOH. The organic phase was dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude of reaction was purified by flash chromatography (FC). Elution with 3:1 EtOAc–petroleum ether gave **12** (110 mg, 65% yield) starting from salviarin (**2**). Elution with 99:1 $CHCl_3$–MeOH gave **13** (130 mg, 73%) from splenolide B (**5**). Elution with 39:1 $CHCl_3$–MeOH gave **15** (108 mg, 58%) from splendidin (**3**).

*4.3.1. Compound **12** (8-epi-salviarin)*

Colorless needles (EtOAc–petroleum ether); mp 232–235 °C; $[\alpha]_D^{18}$ −49.5 (*c* 0.105, $CHCl_3$); lit.[15] mp 233–235 °C; $[\alpha]_D^{18}$ −48 (*c* 0.1, $CHCl_3$); R_f=0.55 (silica gel, 3:2 EtOAc–petroleum ether); IR (KBr) ν_{max} 3152, 3134, 2951, 1792, 1759, 1728, 1507, 1450, 1287, 1221, 1180, 1156, 1032, 993, 875, 808, 735, 702, 602 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (1H, dd, $J_{16,14}$=0.9 Hz, $J_{16,15}$=1.8 Hz, H-16), 7.42 (1H, t, $J_{15,14}$=$J_{15,16}$=1.8 Hz, H-15), 6.40 (1H, dd, $J_{14,15}$=1.8 Hz, $J_{14,16}$=0.9 Hz, H-14), 6.00 (1H, ddt, $J_{2,1\alpha}$=$J_{2,4\beta}$=2.4 Hz, $J_{2,1\beta}$=5.1 Hz, $J_{2,3}$=9.9 Hz, H-2), 5.62 (1H, dtd, $J_{3,1\alpha}$=$J_{3,4\beta}$=2.8 Hz, $J_{3,1\beta}$=1.4 Hz, $J_{3,2}$=9.9 Hz, H-3), 5.35 (1H, dd, $J_{12\beta,11\alpha}$=11.1 Hz, $J_{12\beta,11\beta}$=6.4 Hz, H-12β), 4.18 (1H, dd, $J_{19a,6\beta}$=1.8 Hz, $J_{19a,19b}$=9.1 Hz, pro-*S* H-19a), 4.14 (1H, d, $J_{19b,19a}$=9.9 Hz, pro-*R* H-19b), 2.80 (1H, tdd, $J_{4\beta,1\alpha}$=$J_{4\beta,3}$=2.8 Hz, $J_{4\beta,1\beta}$=2.5 Hz, $J_{4\beta,2}$=2.4 Hz, H-4β), 2.62 (1H, dd, $J_{8\beta,7\alpha}$=11.9 Hz, $J_{8\beta,7\beta}$=3.4 Hz, H-8β), 2.15 (2H, m, H-1β and H-7β), 2.11 (1H, dt, $J_{6\alpha,6\beta}$=13.8 Hz, $J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=3.8 Hz, H-6α), 2.10 (1H, dd, $J_{11\beta,11\alpha}$=14.0 Hz, $J_{11\beta,12\beta}$=6.4 Hz, H-11β), 2.06 (1H, m, H-1α), 1.88 (1H, dd, $J_{11\alpha,11\beta}$=14.0 Hz, $J_{11\alpha,12\beta}$=11.1 Hz, H-11α), 1.87 (1H, dd, $J_{10\beta,1\alpha}$=11.8 Hz, $J_{10\beta,1\beta}$=4.7 Hz, H-10β), 1.85 (1H, dddd, $J_{7\alpha,6\alpha}$=3.8 Hz, $J_{7\alpha,6\beta}$=12.1 Hz, $J_{7\alpha,7\beta}$=14.8 Hz, $J_{7\alpha,8\beta}$=11.9 Hz, H-7α), 1.35 (1H, dddd,

$J_{6\beta,6\alpha}$=13.8 Hz, $J_{6\beta,7\alpha}$=12.1 Hz, $J_{6\beta,7\beta}$=3.9 Hz, $J_{6\beta,19a}$=1.8 Hz, H-6β), 0.87 (3H, s, Me-20); these assignments are in agreement with the partial ^{1}H NMR data previously reported[15] for 8-*epi*-salviarin; ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (s, C-18), 173.3 (s, C-17), 143.9 (d, C-15), 139.6 (d, C-16), 129.2 (d, C-2), 124.1 (s, C-13), 121.1 (d, C-3), 108.4 (d, C-14), 70.1 (t, C-19), 69.9 (d, C-12), 52.3 (d, C-4), 47.5 (d, C-8), 47.3 (d, C-10), 43.3 (t, C-11), 41.6 (s, C-5), 36.1 (s C-9), 34.9 (t, C-6), 22.3 (t, C-1), 20.4 (q, C-20), 18.5 (t, C-7); EIMS *m*/*z* 342 $[M]^+$ (64), 327 (4), 314 (2), 298 (17), 283 (18), 231 (40), 218 (20), 159 (41), 145 (39), 131 (55), 121 (45), 117 (47), 105 (46), 94 (100), 91 (89), 79 (25), 65 (13), 55 (11). Anal.: C 70.08%, H 6.59%; calcd for $C_{20}H_{22}O_5$: C 70.16%, H 6.48%.

4.3.2. Compound **13**

Colorless needles (EtOAc); mp 254–256 °C; $[\alpha]_D^{18}$ −59.8 (*c* 0.194, Me_2CO); R_f=0.42 (silica gel, 95:5 $CHCl_3$–MeOH); IR (KBr) ν_{max} 3488, 3138, 3110, 3070, 2942, 2864, 2580, 1765, 1599, 1502, 1445, 1386, 1165, 1023, 873, 840, 738, 699, 602 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.42 (1H, dt, $J_{16,12}$=$J_{16,14}$=0.9 Hz, $J_{16,15}$=1.8 Hz, H-16), 7.41 (1H, t, $J_{15,14}$=$J_{15,16}$=1.8 Hz, H-15), 6.43 (1H, dd, $J_{14,15}$=1.8 Hz, $J_{14,16}$=0.9 Hz, H-14), 6.03 (1H, ddt, $J_{2,1\alpha}$=$J_{2,4\beta}$=2.2 Hz, $J_{2,1\beta}$=5.9 Hz, $J_{2,3}$=10.0 Hz, H-2), 5.52 (1H, dtd, $J_{3,1\alpha}$=$J_{3,4\beta}$=2.9 Hz, $J_{3,1\beta}$=1.2 Hz, $J_{3,2}$=10.0 Hz, H-3), 4.91 (1H, dd, $J_{12,11\beta}$=4.1 Hz, $J_{12,16}$=0.9 Hz, H-12), 4.38 (1H, d, $J_{11\beta,12}$=4.1 Hz, H-11β), 4.25 (1H, d, $J_{19a,19b}$=9.2 Hz, pro-*R* H-19a), 4.20 (1H, dd, $J_{19b,6\beta}$=1.9 Hz, $J_{19b,19a}$=9.2 Hz, pro-*S* H-19b), 2.73 (2H, m, H-4β and H-8α), 2.18 (1H, ddddd, $J_{1\beta,1\alpha}$=18.4 Hz, $J_{1\beta,2}$=5.9 Hz, $J_{1\beta,3}$=1.2 Hz, $J_{1\beta,4\beta}$=2.4 Hz, $J_{1\beta,10\beta}$=4.6 Hz, H-1β), 2.05 (1H, ddddd, $J_{1\alpha,1\beta}$=18.4 Hz, $J_{1\alpha,2}$=2.2 Hz, $J_{1\alpha,3}$=2.9 Hz, $J_{1\alpha,4\beta}$=2.8 Hz, $J_{1\alpha,10\beta}$=11.8 Hz, H-1α), 2.03 (1H, dddd, $J_{7\beta,6\alpha}$=3.8 Hz, $J_{7\beta,6\beta}$=4.2 Hz, $J_{7\beta,7\alpha}$=14.8 Hz, $J_{7\beta,8\alpha}$=2.9 Hz, H-7β), 1.79 (1H, dddd, $J_{7\alpha,6\alpha}$=4.0 Hz, $J_{7\alpha,6\beta}$=13.9 Hz, $J_{7\alpha,7\beta}$=14.8 Hz, $J_{7\alpha,8\alpha}$=5.6 Hz, H-7α), 1.73 (1H, dd, $J_{10\beta,1\alpha}$=11.8 Hz, $J_{10\beta,1\beta}$=4.6 Hz, H-10β), 1.73 (1H, m, H-6α), 1.20 (1H, dddd, $J_{6\beta,6\alpha}$=14.0 Hz, $J_{6\beta,7\alpha}$=13.9 Hz, $J_{6\beta,7\beta}$=4.2 Hz, $J_{6\beta,19b}$=1.9 Hz, H-6β), 1.03 (3H, s, Me-20); ^{13}C NMR (100 MHz, CD_3OD) δ 179.1 (s, C-17), 177.5 (s, C-18), 144.2 (d, C-15), 140.8 (d, C-16), 130.5 (d, C-2), 126.3 (s, C-13), 121.0 (d, C-3), 109.6 (d, C-14), 87.0 (d, C-11), 70.0 (t, C-19), 67.2 (d, C-12), 53.2 (d, C-4), 46.9 (d C-8), 44.1 (s, C-9), 41.8 (s, C-5), 41.4 (d, C-10), 32.0 (t, C-6), 23.3 (t, C-1), 17.1 (q, C-20), 17.0 (t, C-7); EIMS *m*/*z* 358 $[M]^+$ (30), 340 (20), 304 (35), 262 (100), 233 (23), 203 (6), 187 (5), 171 (4), 157 (14), 139 (38), 129 (13), 117 (16), 105 (14), 97 (29), 91 (28), 81 (8). Anal.: C 67.21%, H 6.29%; calcd for $C_{20}H_{22}O_6$: C 67.02%, H 6.19%.

4.3.3. Compound **15**

Colorless fine needles (MeOH); mp 260–262 °C; $[\alpha]_D^{18}$ −101.5 (*c* 0.481, Me_2CO); R_f=0.20 (silica gel, 95:5 $CHCl_3$–MeOH); IR (KBr) ν_{max} 3492, 3443, 3140, 3039, 2945, 2585, 2550, 2430, 1774, 1759, 1500, 1371, 1296, 1170, 1020, 961, 875, 797, 702, 607 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (1H, dt, $J_{16,12}$=$J_{16,14}$=0.8 Hz, $J_{16,15}$=1.9 Hz, H-16), 7.45 (1H, t, $J_{15,14}$=$J_{15,16}$=1.9 Hz, H-15), 6.49 (1H, dd, $J_{14,15}$=1.9 Hz, $J_{14,16}$=0.8 Hz, H-14), 5.91 (1H, ddd, $J_{2,1\alpha}$=1.8 Hz, $J_{2,3}$=10.0 Hz, $J_{2,4\beta}$=2.6 Hz, H-2), 5.62 (1H, d, $J_{11\beta,12}$=3.6 Hz, H-11β), 5.51 (1H, ddd, $J_{3,1\alpha}$=2.0 Hz, $J_{3,2}$=10.0 Hz, $J_{3,4\beta}$=3.2 Hz, H-3), 4.97 (1H, dd, $J_{12,11\beta}$=3.6 Hz, $J_{12,16}$=0.8 Hz, H-12), 4.38 (1H, dddd, $J_{1\alpha,2}$=1.8 Hz, $J_{1\alpha,3}$=2.0 Hz, $J_{1\alpha,4\beta}$=3.2 Hz, $J_{1\alpha,10\beta}$=10.4 Hz, H-1α), 4.35 (1H, d, $J_{19a,19b}$=9.2 Hz, pro-*R* H-19a), 4.20 (1H, dd, $J_{19b,6\beta}$=1.8 Hz, $J_{19b,19a}$=9.2 Hz, pro-*S* H-19b), 2.80 (1H, td, $J_{4\beta,1\alpha}$=$J_{4\beta,3}$=3.2 Hz, $J_{4\beta,2}$=2.6 Hz, H-4β), 2.66 (1H, ddd, $J_{8\alpha,6\alpha}$=1.2 Hz, $J_{8\alpha,7\alpha}$=5.4 Hz, $J_{8\alpha,7\beta}$=2.4 Hz, H-8α), 1.93 (1H, dddd, $J_{7\beta,6\alpha}$=2.7 Hz, $J_{7\beta,6\beta}$=4.0 Hz, $J_{7\beta,7\alpha}$=14.8 Hz, $J_{7\beta,8\alpha}$=2.4 Hz, H-7β), 1.82 (1H, dddd, $J_{7\alpha,6\alpha}$=3.8 Hz, $J_{7\alpha,6\beta}$=13.8 Hz, $J_{7\alpha,7\beta}$=14.8 Hz, $J_{7\alpha,8\alpha}$=5.4 Hz, H-7α), 1.75 (1H, d, $J_{10\beta,1\alpha}$=10.4 Hz, H-10β), 1.63 (1H, dddd, $J_{6\alpha,6\beta}$=14.0 Hz, $J_{6\alpha,7\alpha}$=3.8 Hz, $J_{6\alpha,7\beta}$=2.7 Hz, $J_{6\alpha,8\alpha}$=1.2 Hz, H-6α), 1.21 (1H, dddd, $J_{6\beta,6\alpha}$=14.0 Hz, $J_{6\beta,7\alpha}$=13.8 Hz, $J_{6\beta,7\beta}$=4.0 Hz, $J_{6\beta,19b}$=1.8 Hz, H-6β), 1.20 (3H, s, Me-20); ^{13}C NMR (100 MHz, CD_3OD) δ 180.5 (s, C-17), 177.5 (s, C-18), 144.3 (d, C-15), 141.6 (d, C-16), 137.6 (d, C-2), 127.1 (s, C-13), 120.7 (d, C-3), 110.8 (d, C-14), 88.7 (d, C-11), 70.8 (t, C-19), 68.0 (d, C-12), 66.7 (d, C-1), 53.8 (d, C-4), 47.8 (d, C-8), 46.8 (d, C-10), 44.7 (s, C-9), 42.9 (s, C-5), 31.9 (t, C-6), 17.7 (q, C-20), 17.3 (t, C-7); EIMS *m*/*z* 374 $[M]^+$ (3), 356 (7), 278 (61), 259 (25), 203 (33), 159 (20), 145 (41), 135 (83), 117 (38), 97 (100), 91 (42), 81 (15), 69 (14). Anal.: C 64.27%, H 6.02%; calcd for $C_{20}H_{22}O_7$: C 64.16%, H 5.92%.

4.4. 8-*epi*-Splenolide B (17) from splenolide B (5)

To a solution of **5** (50 mg, 0.125 mmol) in CH_2Cl_2 (0.5 mL), 170 μL (173 mg, 1.14 mmol) of DBU were added in one portion via syringe. The solution was left under Ar for 72 h at room temperature. Then the reaction mixture was quenched by addition of 1 N HCl (1 mL) and was extracted with $CHCl_3$ (4×5 mL). The collected organic phase was washed with brine and dried over Na_2SO_4. Removal of the solvent by distillation at reduced pressure followed by FC (1–5% acetone–CH_2Cl_2 in gradient) successively yielded **17** (20 mg, 40%), **4** (7 mg, 15%), and **13** (4 mg, 10%).

Compound ***17***: colorless rectangular plates (EtOAc–petroleum ether); mp 201–203 °C; $[\alpha]_D^{18}$ −84.4 (*c* 0.263, $CHCl_3$); R_f=0.35 (silica gel, 5% acetone–CH_2Cl_2); IR (KBr) ν_{max} 3138, 3036, 2918, 1765, 1740, 1730, 1503, 1385, 1249, 1237, 1190, 1176, 1153, 1024, 1011, 967, 876, 798, 702, 599 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (1H, ddd, $J_{16,12\beta}$=1.8 Hz, $J_{16,14}$=0.9 Hz, $J_{16,15}$=1.9 Hz, H-16), 7.42 (1H, t, $J_{15,14}$=$J_{15,16}$=1.9 Hz, H-15), 6.55 (1H, dd, $J_{14,15}$=1.9 Hz, $J_{14,16}$=0.9 Hz, H-14), 5.94 (1H, ddt, $J_{2,1\alpha}$=$J_{2,4\beta}$=2.5 Hz, $J_{2,1\beta}$=5.8 Hz, $J_{2,3}$=10.0 Hz, H-2), 5.60 (1H, dtd, $J_{3,1\alpha}$=$J_{3,4\beta}$=2.7 Hz, $J_{3,1\beta}$=1.4 Hz, $J_{3,2}$=10.0 Hz, H-3), 5.30 (1H, dd, $J_{12\beta,11\alpha}$=2.3 Hz, $J_{12\beta,16}$=1.8 Hz, H-12β), 5.12 (1H, d, $J_{11\alpha,12\beta}$=2.3 Hz, H-11α), 4.13 (2H, br s, H_2-19), 2.81 (1H, tdd, $J_{4\beta,1\alpha}$=$J_{4\beta,3}$=2.7 Hz, $J_{4\beta,1\beta}$=2.4 Hz, $J_{4\beta,2}$=2.5 Hz, H-4β), 2.78 (1H, dd, $J_{8\beta,7\alpha}$=12.3 Hz, $J_{8\beta,7\beta}$=3.9 Hz, H-8β), 2.27 (1H, dtd, $J_{7\beta,6\alpha}$=3.5 Hz, $J_{7\beta,6\beta}$=$J_{7\beta,8\beta}$=3.9 Hz, $J_{7\beta,7\alpha}$=14.8 Hz, H-7β), 2.16 (3H, s, 11β-OAc), 2.09 (1H, ddd, $J_{6\alpha,6\beta}$=13.8 Hz, $J_{6\alpha,7\alpha}$=3.2 Hz, $J_{6\alpha,7\beta}$=3.5 Hz, H-6α), 2.04 (1H, dd, $J_{10\beta,1\alpha}$=11.8 Hz, $J_{10\beta,1\beta}$=6.6 Hz, H-10β), 1.90 (2H, m, H-1α and H-1β), 1.72 (1H, dddd, $J_{7\alpha,6\alpha}$=3.2 Hz, $J_{7\alpha,6\beta}$=13.8 Hz, $J_{7\alpha,7\beta}$=14.8 Hz, $J_{7\alpha,8\beta}$=12.3 Hz, H-7α), 1.34 (1H, td, $J_{6\beta,6\alpha}$=$J_{6\beta,7\alpha}$=13.8 Hz, $J_{6\beta,7\beta}$=3.9 Hz, H-6β), 0.76 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2 (s, C-18), 170.7 (s, C-17), 169.9 (s, 11-OCOCH_3), 144.0 (d, C-15), 139.4 (d, C-16), 128.9 (d, C-2), 124.1 (s, C-13), 120.9 (d, C-3), 108.3 (d, C-14), 78.6 (d, C-12), 74.2 (d, C-11), 69.6 (t, C-19), 52.4 (d, C-4), 43.2 (d, C-8), 40.9 (s, C-5), 39.4 (d, C-10), 39.2 (s, C-9), 34.7 (t, C-6), 21.8 (t, C-1), 21.0 (q, 11-OCOCH_3), 18.6 (t, C-7), 15.2 (q, C-20); EIMS *m*/*z* 400 $[M]^+$ (1), 358 (1), 340 (63), 325 (100), 312 (1), 297 (4), 244 (5), 189 (8), 143 (10), 129 (13), 117 (11), 105 (11), 95 (22), 91 (27), 81 (10), 55 (7). Anal.: C 66.08%, H 6.19%; calcd for $C_{22}H_{24}O_7$: C 65.99%, H 6.04%.

4.5. 8-*epi*-Splendidin (18) from splendidin (3)

To a solution of **3** (50 mg, 0.109 mmol) in anhydrous CH_2Cl_2 (0.5 mL), 150 μL (153 mg, 1.00 mmol) of DBU were added in one portion via syringe. The solution was left under Ar for 24 h at room temperature. Workup as described above for **17** followed by FC (49:1 CH_2Cl_2–acetone as eluent) gave **18** (35 mg, 70%) and unreacted **3** (7.5 mg, 15%).

Compound ***18***: colorless rectangular plates (EtOAc–*n*-pentane); mp 232–235 °C; $[\alpha]_D^{18}$ −142.1 (*c* 0.151, $CHCl_3$); R_f=0.30 (silica gel, 49:1 CH_2Cl_2–acetone); IR (KBr) ν_{max} 3139, 3109, 3015, 2922, 1771, 1734, 1370, 1238, 1175, 1160, 1026, 965, 876, 607 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (1H, dt, $J_{16,12\beta}$=$J_{16,14}$=0.8 Hz, $J_{16,15}$=1.7 Hz, H-16), 7.41 (1H, t, $J_{15,14}$=$J_{15,16}$=1.7 Hz, H-15), 6.56 (1H, dd, $J_{14,15}$=1.7 Hz, $J_{14,16}$=0.8 Hz, H-14), 5.71 (1H, ddd, $J_{2,1\alpha}$=2.8 Hz, $J_{2,3}$=10.0 Hz, $J_{2,4\beta}$=1.6 Hz, H-2), 5.69 (1H, dd, $J_{3,2}$=10.0 Hz, $J_{3,4\beta}$=1.6 Hz, H-3), 5.50 (1H, dd, $J_{1\alpha,2}$=2.8 Hz, $J_{1\alpha,4\beta}$=1.6 Hz, $J_{1\alpha,10\beta}$=10.8 Hz, H-1α), 5.40 (1H, dd, $J_{12\beta,11\alpha}$=0.4 Hz, $J_{12\beta,16}$=0.8 Hz, H-12β), 5.10 (1H, d, $J_{11\alpha,12\beta}$=0.4 Hz, H-11α), 4.23 (1H, d,

$J_{19a,19b}$=9.2 Hz, pro-*R* H-19a), 4.18 (1H, dd, $J_{19b,6\beta}$=1.7 Hz, $J_{19b,19a}$=9.2 Hz, pro-*S* H-19b), 2.89 (1H, q, $J_{4\beta,1\alpha}$=$J_{4\beta,2}$=$J_{4\beta,3}$=1.6 Hz, H-4β), 2.88 (1H, dd, $J_{8\beta,7\alpha}$=12.6 Hz, $J_{8\beta,7\beta}$=3.8 Hz, H-8β), 2.45 (1H, d, $J_{10\beta,1\alpha}$=10.8 Hz, H-10β), 2.33 (1H, dddd, $J_{7\beta,6\alpha}$=3.2 Hz, $J_{7\beta,6\beta}$=3.6 Hz, $J_{7\beta,7\alpha}$=14.8 Hz, $J_{7\beta,8\beta}$=3.8 Hz, H-7β), 2.22 (3H, s, 11β-OAc), 2.10 (3H, s, 1β-OAc), 2.07 (1H, dt, $J_{6\alpha,6\beta}$=13.8 Hz, $J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=3.2 Hz, H-6α), 1.71 (1H, dddd, $J_{7\alpha,6\alpha}$=3.2 Hz, $J_{7\alpha,6\beta}$=14.0 Hz, $J_{7\alpha,7\beta}$=14.8 Hz, $J_{7\alpha,8\beta}$=12.6 Hz, H-7α), 1.42 (1H, dddd, $J_{6\beta,6\alpha}$=13.8 Hz, $J_{6\beta,7\alpha}$=14.0 Hz, $J_{6\beta,7\beta}$=3.6 Hz, $J_{6\beta,19b}$=1.7 Hz, H-6β), 0.80 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8 (s, C-18), 170.6 (s, 1-OCOCH$_3$), 170.3 (s, 11-OCOCH$_3$), 169.9 (s, C-17), 143.9 (d, C-15), 138.9 (d, C-16), 130.7 (d, C-2), 124.9 (s, C-13), 122.4 (d, C-3), 108.0 (d, C-14), 79.2 (d, C-12), 76.2 (d, C-11), 69.8 (t, C-19), 68.2 (d, C-1), 52.2 (d, C-4), 43.1 (d, C-8), 42.0 (d, C-10), 41.7 (s, C-5), 39.2 (s, C-9), 34.2 (t, C-6), 21.4 (q, 1-OCOCH$_3$), 21.2 (q, 11-OCOCH$_3$), 18.2 (t, C-7), 15.1 (q, C-20); EIMS *m*/*z* 458 $[M]^+$ (1), 398 (39), 356 (100), 323 (52), 203 (27), 189 (30), 176 (18), 161 (10), 129 (10), 110 (8), 95 (20), 91 (12), 81 (10). Anal.: C 62.59%, H 5.89%; calcd for $C_{24}H_{26}O_9$: C 62.88%, H 5.72%.

4.6. Preparation of compound 19 from salvisplendin C (7)

A solution of **7** (100 mg, 0.257 mmol) in MeOH (1 mL) was treated with an excess of CH_2N_2 in Et_2O at 0 °C. After 1 h, the solvent was removed in vacuo and pure pyrazoline derivative **19** was recovered as an amorphous solid. $[\alpha]_D^{18}$ −26.4 (*c* 0.106, $CHCl_3$); R_f=0.30 (silica gel, 3:2 EtOAc–petroleum ether); IR (KBr) ν_{max} 3463, 3130, 2942, 1766, 1543, 1489, 1434, 1374, 1241, 1185, 1018, 969, 925, 875, 810, 755, 602 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (1H, dd, $J_{16,14}$=1.0 Hz, $J_{16,15}$=1.8 Hz, H-16), 7.32 (1H, t, $J_{15,14}$=$J_{15,16}$=1.8 Hz, H-15), 6.34 (1H, dd, $J_{14,15}$=1.8 Hz, $J_{14,16}$=1.0 Hz, H-14), 5.68 (1H, dd, $J_{12,11a}$=6.7 Hz, $J_{12,11b}$=5.1 Hz, H-12), 5.45 (1H, d, $J_{19a,19b}$=9.0 Hz, pro-*R* H-19a), 4.63 (1H, d, $J_{3'\beta,3'\alpha}$=16.8 Hz, H-3′β), 4.27 (1H, dd, $J_{19b,6\beta}$=2.0 Hz, $J_{19b,19a}$=9.0 Hz, pro-*S* H-19b), 4.23 (1H, dd, $J_{3'\alpha,3\alpha}$=6.0 Hz, $J_{3'\alpha,3'\beta}$=16.8 Hz, H-3′α), 4.23 (1H, m, H-7β), 2.74 (1H, ddd, $J_{6\beta,6\alpha}$=14.9 Hz, $J_{6\beta,7\beta}$=3.7 Hz, $J_{6\beta,19b}$=2.0 Hz, H-6β), 2.58 (1H, dd, $J_{6\alpha,6\beta}$=14.9 Hz, $J_{6\alpha,7\beta}$=2.6 Hz, H-6α), 2.29 (1H, m, H-1β), 2.26 (1H, m, H-1α), 2.18 (1H, ddd, $J_{3\alpha,2\alpha}$=6.3 Hz, $J_{3\alpha,2\beta}$=13.1 Hz, $J_{3\alpha,3'\alpha}$=6.0 Hz, H-3α), 2.05 (1H, dd, $J_{11a,11b}$=15.8 Hz, $J_{11a,12}$=6.7 Hz, H-11a), 1.97 (3H, s, 11-OAc), 1.79 (1H, qd, $J_{8\beta,7\beta}$=2.9 Hz, $J_{8\beta,17}$=7.0 Hz, H-8β), 1.77 (1H, dd, $J_{11b,11a}$=15.8 Hz, $J_{11b,12}$=5.1 Hz, H-11b), 1.67 (1H, dddd, $J_{2\alpha,1\alpha}$=2.7 Hz, $J_{2\alpha,1\beta}$=3.1 Hz, $J_{2\alpha,2\beta}$=13.4 Hz, $J_{2\alpha,3\alpha}$=6.3 Hz, H-2α), 1.37 (1H, dd, $J_{10\beta,1\alpha}$=11.9 Hz, $J_{10\beta,1\beta}$=2.5 Hz, H-10β), 1.16 (3H, d, $J_{17,8\beta}$=7.0 Hz, Me-17), 0.88 (3H, s, Me-20), 0.33 (1H, qd, $J_{2\beta,1\alpha}$=$J_{2\beta,2\alpha}$=$J_{2\beta,3\alpha}$=13.1 Hz, $J_{2\beta,1\beta}$=4.5 Hz, H-2β); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (s, C-18), 169.9 (s, 12-OCOCH$_3$), 143.4 (d, C-15), 140.1 (d, C-16), 125.7 (s, C-13), 108.7 (d, C-14), 95.7 (s, C-4), 81.8 (t, C-3′), 72.7 (d, C-7), 71.2 (t, C-19), 64.5 (d, C-12), 45.5 (s, C-5), 44.7 (d, C-10), 42.4 (t, C-11), 40.3 (d, C-8), 39.4 (s, C-9), 38.2 (t, C-6), 35.4 (d, C-3), 28.9 (t, C-2), 21.2 (q, 11-OCOCH$_3$), 19.4 (q, C-20), 18.7 (t, C-1), 12.6 (q, C-17); EIMS *m*/*z* 430 $[M]^+$ (3), 370 (1), 342 (2), 300 (4), 277 (6), 247 (19), 231 (15), 218 (33), 190 (35), 175 (29), 121 (28), 105 (28), 94 (100), 91 (34), 81 (27), 55 (21). Anal.: C 63.95%, H 7.19%, N 6.42%; calcd for $C_{23}H_{30}N_2O_6$: C 64.17%, H 7.02%, N 6.51%.

4.7. Procedure for the acetylation of compounds 9, 13, 15, and 19

Treatment of **9** (20 mg, 0.052 mmol), **13** (50 mg, 0.140 mmol), and **15** (50 mg, 0.134 mmol) with Ac_2O–pyridine (1:2, 1 mL) for 24 h at room temperature followed by standard workup and purification by FC yielded quantitatively the acetyl derivatives **21** (19:1 CH_2Cl_2–acetone as eluent), **14** (elution with 32:1 CH_2Cl_2–acetone), and **16** (elution with 19:1 CH_2Cl_2–acetone), respectively. Compound **19** (20 mg, 0.052 mmol) was treated with Ac_2O–pyridine (1:2, 3 mL) for one week at 40 °C. After standard workup the residue of the reaction was chromatographed (Si gel 230–400 mesh column, 20 g, 4:1 petroleum ether–EtOAc as eluent) yielding **20** (13.5 mg, 55%).

4.7.1. Compound *14*

Colorless prisms (EtOAc–petroleum ether); mp 130–132 °C; $[\alpha]_D^{18}$ −30.6 (*c* 0.435, $CHCl_3$); R_f=0.40 (silica gel, 99:1 CH_2Cl_2–acetone); IR (KBr) ν_{max} 3134, 3033, 2937, 2263, 1777, 1758, 1738, 1504, 1372, 1235, 1158, 1023, 962, 874, 799, 756, 741, 697, 603 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (1H, dd, $J_{16,14}$=0.7 Hz, $J_{16,15}$=1.8 Hz, H-16), 7.41 (1H, t, $J_{15,14}$=$J_{15,16}$=1.8 Hz, H-15), 6.49 (1H, dd, $J_{14,15}$=1.8 Hz, $J_{14,16}$=0.7 Hz, H-14), 5.99 (1H, ddt, $J_{2,1\alpha}$=$J_{2,4\beta}$=2.4 Hz, $J_{2,1\beta}$=4.8 Hz, $J_{2,3}$=10.2 Hz, H-2), 5.91 (1H, d, $J_{12,11\beta}$=3.1 Hz, H-12), 5.60 (1H, dddd, $J_{3,1\alpha}$=3.2 Hz, $J_{3,1\beta}$=1.2 Hz, $J_{3,2}$=10.2 Hz, $J_{3,4\beta}$=3.4 Hz, H-3), 4.49 (1H, d, $J_{11\beta,12}$=3.1 Hz, H-11β), 4.18 (1H, dd, $J_{19a,6\beta}$=1.6 Hz, $J_{19a,19b}$=9.0 Hz, pro-*S* H-19a), 4.14 (1H, d, $J_{19b,19a}$=9.0 Hz, pro-*R* H-19b), 2.73 (1H, tt, $J_{4\beta,1\alpha}$=$J_{4\beta,3}$=3.4 Hz, $J_{4\beta,1\beta}$=$J_{4\beta,2}$=2.4 Hz, H-4β), 2.20 (1H, dddd, $J_{7\beta,6\alpha}$=3.8 Hz, $J_{7\beta,6\beta}$=4.0 Hz, $J_{7\beta,7\alpha}$=14.5 Hz, $J_{7\beta,8\alpha}$=2.8 Hz, H-7β), 2.14 (1H, ddddd, $J_{1\alpha,1\beta}$=18.0 Hz, $J_{1\alpha,2}$=2.4 Hz, $J_{1\alpha,3}$=3.2 Hz, $J_{1\alpha,4\beta}$=3.4 Hz, $J_{1\alpha,10\beta}$=11.8 Hz, H-1α), 2.10 (2H, m, H-1β and H-8α), 2.07 (3H, s, 12-OAc), 1.77 (1H, dtd, $J_{6\alpha,6\beta}$=14.0 Hz, $J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=3.8 Hz, $J_{6\alpha,8\alpha}$=1.4 Hz, H-6α), 1.73 (1H, dd, $J_{10\beta,1\alpha}$=11.8 Hz, $J_{10\beta,1\beta}$=5.2 Hz, H-10β), 1.64 (1H, dddd, $J_{7\alpha,6\alpha}$=3.8 Hz, $J_{7\alpha,6\beta}$=14.0 Hz, $J_{7\alpha,7\beta}$=14.5 Hz, $J_{7\alpha,8\alpha}$=5.4 Hz, H-7α), 1.21 (1H, tdd, $J_{6\beta,6\alpha}$=$J_{6\beta,7\alpha}$=14.0 Hz, $J_{6\beta,7\beta}$=4.0 Hz, $J_{6\beta,19a}$=1.6 Hz, H-6β), 1.08 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3 (s, C-17), 175.1 (s, C-18), 170.0 (s, 12-OCOCH$_3$), 144.0 (d, C-15), 141.9 (d, C-16), 128.9 (d, C-2), 121.0 (d, C-3), 119.7 (s, C-13), 109.9 (d, C-14), 84.1 (d, C-11), 68.6 (t, C-19), 67.1 (d, C-12), 52.1 (d, C-4), 45.5 (d, C-8), 42.7 (s, C-9), 40.9 (s, C-5), 40.8 (d, C-10), 31.0 (t, C-6), 22.7 (t, C-1), 21.1 (q, 12-OCOCH$_3$), 16.6 (q, C-20), 16.5 (t, C-7); EIMS *m*/*z* 400 $[M]^+$ (2), 358 (30), 340 (19), 304 (32), 262 (100), 233 (24), 203 (7), 187 (6), 157 (16), 139 (35), 129 (14), 117 (18), 105 (14), 97 (28), 91 (29), 81 (8). Anal.: C 66.15%, H 6.01%; calcd for $C_{22}H_{24}O_7$: C 65.99%, H 6.04%.

4.7.2. Compound *16*

Amorphous, white powder; $[\alpha]_D^{18}$ −78.9 (*c* 0.503, $CHCl_3$); R_f=0.35 (silica gel, 99:1 CH_2Cl_2–acetone); IR (KBr) ν_{max} 3146, 2947, 1781, 1740, 1503, 1373, 1234, 1168, 1024, 962, 915, 875, 800, 756, 603 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (1H, dd, $J_{16,14}$=0.9 Hz, $J_{16,15}$=1.8 Hz, H-16), 7.39 (1H, t, $J_{15,14}$=$J_{15,16}$=1.8 Hz, H-15), 6.42 (1H, dd, $J_{14,15}$=1.8 Hz, $J_{14,16}$=0.9 Hz, H-14), 5.89 (1H, d, $J_{12,11\beta}$=6.3 Hz, H-12), 5.75 (1H, ddd, $J_{2,1\alpha}$=2.0 Hz, $J_{2,3}$=10.0 Hz, $J_{2,4\beta}$=2.6 Hz, H-2), 5.69 (1H, ddd, $J_{3,1\alpha}$=2.0 Hz, $J_{3,2}$=10.0 Hz, $J_{3,4\beta}$=2.6 Hz, H-3), 5.65 (1H, ddt, $J_{1\alpha,2}$=$J_{1\alpha,3}$=2.0 Hz, $J_{1\alpha,4\beta}$=2.6 Hz, $J_{1\alpha,10\beta}$=10.8 Hz, H-1α), 5.18 (1H, d, $J_{11\beta,12}$=6.3 Hz, H-11β), 4.24 (1H, d, $J_{19a,19b}$=9.2 Hz, pro-*R* H-19a), 4.21 (1H, dd, $J_{19b,6\beta}$=1.6 Hz, $J_{19b,19a}$=9.2 Hz, pro-*S* H-19b), 2.81 (1H, q, $J_{4\beta,1\alpha}$=$J_{4\beta,2}$=$J_{4\beta,3}$=2.6 Hz, H-4β), 2.32 (1H, ddd, $J_{8\alpha,6\alpha}$=1.2 Hz, $J_{8\alpha,7\alpha}$=4.4 Hz, $J_{8\alpha,7\beta}$=2.8 Hz, H-8α), 2.17 (3H, s, 1β-OAc), 2.11 (1H, d, $J_{10\beta,1\alpha}$=10.8 Hz, H-10β), 2.10 (1H, dddd, $J_{7\beta,6\alpha}$=3.6 Hz, $J_{7\beta,6\beta}$=4.0 Hz, $J_{7\beta,7\alpha}$=14.4 Hz, $J_{7\beta,8\alpha}$=2.8 Hz, H-7β), 2.02 (3H, s, 12-OAc), 1.76 (1H, dtd, $J_{6\alpha,6\beta}$=13.8 Hz, $J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=3.6 Hz, $J_{6\alpha,8\alpha}$=1.2 Hz, H-6α), 1.71 (1H, dddd, $J_{7\alpha,6\alpha}$=3.6 Hz, $J_{7\alpha,6\beta}$=13.6 Hz, $J_{7\alpha,7\beta}$=14.4 Hz, $J_{7\alpha,8\alpha}$=4.4 Hz, H-7α), 1.27 (1H, dddd, $J_{6\beta,6\alpha}$=13.8 Hz, $J_{6\beta,7\alpha}$=13.6 Hz, $J_{6\beta,7\beta}$=4.0 Hz, $J_{6\beta,19b}$=1.6 Hz, H-6β), 1.11 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8 (s, C-17), 173.6 (s, C-18), 169.8 (s, 1β-OCOCH$_3$), 169.2 (s, 12-OCOCH$_3$), 143.9 (d, C-15), 142.0 (d, C-16), 131.0 (d, C-2), 122.4 (d, C-3), 120.6 (s, C-13), 109.3 (d, C-14), 83.7 (d, C-11), 68.7 (t, C-19), 68.3 (d, C-1), 65.3 (d, C-12), 51.9 (d, C-4), 45.2 (d, C-8), 43.0 (d, C-10), 42.5 (s, C-9), 41.5 (s, C-5), 30.4 (t, C-6), 21.3 (q, 1β-OCOCH$_3$), 21.0 (q, 12-OCOCH$_3$), 17.2 (q, C-20), 16.3 (t, C-7); EIMS *m*/*z* 458 $[M]^+$ (1), 416 (3), 398 (100), 356 (85), 320 (20), 277 (17), 259 (57), 231 (17), 222 (17), 203 (28), 157 (20), 143 (44), 139 (48), 135 (55), 117 (26), 105 (14), 97 (420), 91 (28), 81 (14). Anal.: C 62.69%, H 5.84%; calcd for $C_{24}H_{26}O_9$: C 62.88%, H 5.72%.

*4.7.3. Compound **20***

Amorphous, white solid; $[\alpha]_D^{20}$ −38.2 (*c* 0.204, $CHCl_3$); R_f=0.55 (silica gel, 3:2 EtOAc–petroleum ether); IR (KBr) ν_{max} 3135, 2947, 1774, 1738, 1545, 1434, 1371, 1234, 1180, 1020, 976, 875, 603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (2H, m, H-15 and H-16), 6.33 (1H, dd, $J_{14,15}$=2.0 Hz, $J_{14,16}$=1.0 Hz, H-14), 5.67 (1H, dd, $J_{12,11a}$=7.4 Hz, $J_{12,11b}$=4.7 Hz, H-12), 5.45 (1H, ddd, $J_{7\beta,6\alpha}$=2.5 Hz, $J_{7\beta,6\beta}$=4.0 Hz, $J_{7\beta,8\beta}$=3.5 Hz, H-7β), 4.99 (1H, d, $J_{19a,19b}$=9.3 Hz, pro-*R* H-19a), 4.66 (1H, d, $J_{3'\beta,3'\alpha}$=17.0 Hz, H-3′β), 4.31 (1H, dd, $J_{19b,6\beta}$=2.1 Hz, $J_{19b,19a}$=9.3 Hz, pro-*S* H-19b), 4.24 (1H, dd, $J_{3'\alpha,3\alpha}$=6.0 Hz, $J_{3'\alpha,3'\beta}$=17.0 Hz, H-3′α), 2.86 (1H, ddd, $J_{6\beta,6\alpha}$=15.6 Hz, $J_{6\beta,7\beta}$=4.0 Hz, $J_{6\beta,19b}$=2.1 Hz, H-6β), 2.54 (1H, dd, $J_{6\alpha,6\beta}$=15.6 Hz, $J_{6\alpha,7\beta}$=2.5 Hz, H-6α), 2.20 (1H, ddd, $J_{3\alpha,2\alpha}$=6.6 Hz, $J_{3\alpha,2\beta}$=12.7 Hz, $J_{3\alpha,3'\alpha}$=6.0 Hz, H-3α), 2.12 (3H, s, 7α-OAc), 2.07 (1H, dd, $J_{11a,11b}$=16.0 Hz, $J_{11a,12}$=7.4 Hz, H-11a), 1.97 (3H, s, 11-OAc), 1.95 (1H, qd, $J_{8\beta,7\beta}$=3.5 Hz, $J_{8\beta,17}$=7.0 Hz, H-8β), 1.78 (1H, dd, $J_{11b,11a}$=16.0 Hz, $J_{11b,12}$=4.7 Hz, H-11b), 1.71 (1H, dddd, $J_{2\alpha,1\alpha}$=2.3 Hz, $J_{2\alpha,1\beta}$=3.5 Hz, $J_{2\alpha,2\beta}$=13.5 Hz, $J_{2\alpha,3\alpha}$=6.6 Hz, H-2α), 1.42 (1H, dd, $J_{10\beta,1\alpha}$=12.0 Hz, $J_{10\beta,1\beta}$=2.4 Hz, H-10β), 1.35 (1H, dddd, $J_{1\beta,1\alpha}$=13.0 Hz, $J_{1\beta,2\alpha}$=3.5 Hz, $J_{1\beta,2\beta}$=4.0 Hz, $J_{1\beta,10\beta}$=2.4 Hz, H-1β), 1.26 (1H, dddd, $J_{1\alpha,1\beta}$=13.0 Hz, $J_{1\alpha,2\alpha}$=2.3 Hz, $J_{1\alpha,2\beta}$=12.6 Hz, $J_{1\alpha,10\beta}$=12.0 Hz, H-1α), 1.04 (3H, d, $J_{17,8\beta}$=7.0 Hz, Me-17), 0.84 (3H, s, Me-20), 0.40 (1H, dddd, $J_{2\beta,1\alpha}$=12.6 Hz, $J_{2\beta,1\beta}$=4.0 Hz, $J_{2\beta,2\alpha}$=13.5 Hz, $J_{2\beta,3\alpha}$=12.7 Hz, H-2β); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (s, C-18), 169.82 (s, 12-OCOCH_3), 169.78 (s, 7-OCOCH_3), 143.5 (d, C-15), 140.1 (d, C-16), 125.6 (s, C-13), 108.6 (d, C-14), 95.2 (s, C-4), 81.8 (t, C-3′), 73.4 (d, C-7), 70.6 (t, C-19), 64.4 (d, C-12), 45.3 (s, C-5), 44.4 (d, C-10), 42.0 (t, C-11), 39.4 (d, C-8), 39.3 (s, C-9), 35.8 (t, C-6), 35.4 (d, C-3), 28.8 (t, C-2), 21.4 (q, 7-OCOCH_3), 21.2 (q, 12-OCOCH_3), 19.1 (q, C-20), 18.7 (t, C-1), 12.0 (q, C-17); EIMS *m*/*z* 472 $[M]^+$ (1), 430 (1), 412 (1), 402 (3), 342 (7), 260 (30), 249 (37), 231 (49), 185 (33), 172 (42), 157 (53), 119 (48), 111 (47), 94 (100), 81 (31), 55 (18). Anal.: C 63.63%, H 6.71%, N 5.79%; calcd for $C_{25}H_{32}N_2O_7$: C 63.54%, H 6.83%, N 5.93%.

*4.7.4. Compound **21***

Colorless fine needles (EtOAc–*n*-pentane); mp 222–224 °C; $[\alpha]_D^{20}$ −54.6 (*c* 0.509, $CHCl_3$); R_f=0.42 (silica gel, 95:5 CH_2Cl_2–acetone); IR (KBr) ν_{max} 3127, 3018, 2966, 2928, 1782, 1741, 1542, 1500, 1459, 1373, 1240, 1202, 1151, 1045, 1025, 965, 910, 874, 797, 719, 600 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (1H, t, $J_{15,14}$=$J_{15,16}$=1.5 Hz, H-15), 7.34 (1H, dd, $J_{16,14}$=0.8 Hz, $J_{16,15}$=1.5 Hz, H-16), 6.32 (1H, dd, $J_{14,15}$=1.5 Hz, $J_{14,16}$=0.8 Hz, H-14), 4.95 (1H, dd, $J_{12\beta,11\alpha}$=9.9 Hz, $J_{12\beta,11\beta}$=7.1 Hz, H-12β), 4.91 (1H, d, $J_{3'\beta,3'\alpha}$=17.3 Hz, H-3′β), 4.50 (1H, d, $J_{19a,19b}$=9.4 Hz, pro-*R* H-19a), 4.32 (1H, dd, $J_{19b,6\beta}$=2.0 Hz, $J_{19b,19a}$=9.4 Hz, pro-*S* H-19b), 4.30 (1H, dd, $J_{3'\alpha,3\alpha}$=6.1 Hz, $J_{3'\alpha,3'\beta}$=17.3 Hz, H-3′α), 4.14 (1H, ddd, $J_{2\beta,1\alpha}$=11.4 Hz, $J_{2\beta,1\beta}$=4.3 Hz, $J_{2\beta,3\alpha}$=10.4 Hz, H-2β), 2.76 (1H, dddd, $J_{6\beta,6\alpha}$=14.0 Hz, $J_{6\beta,7\alpha}$=13.4 Hz, $J_{6\beta,7\beta}$=3.3 Hz, $J_{6\beta,19b}$=2.0 Hz, H-6β), 2.31 (1H, dd, $J_{3\alpha,2\beta}$=10.4 Hz, $J_{3\alpha,3'\alpha}$=6.1 Hz, H-3α), 2.25 (1H, dd, $J_{11\beta,11\alpha}$=13.2 Hz, $J_{11\beta,12\beta}$=7.1 Hz, H-11β), 2.12 (2H, m, H-6α and H-7β), 2.08 (3H, s, 2α-OAc), 1.89 (1H, ddd, $J_{1\beta,1\alpha}$=12.7 Hz, $J_{1\beta,2\beta}$=4.3 Hz, $J_{1\beta,10\beta}$=2.2 Hz, H-1β), 1.83 (1H, dd, $J_{11\alpha,11\beta}$=13.2 Hz, $J_{11\alpha,12\beta}$=9.9 Hz, H-11α), 1.75 (1H, ddd, $J_{7\alpha,6\alpha}$=3.8 Hz, $J_{7\alpha,6\beta}$=13.4 Hz, $J_{7\alpha,7\beta}$=15.5 Hz, H-7α), 1.70 (1H, dd, $J_{10\beta,1\alpha}$=13.0 Hz, $J_{10\beta,1\beta}$=2.2 Hz, H-10β), 1.42 (1H, ddd, $J_{1\alpha,1\beta}$=12.7 Hz, $J_{1\alpha,2\beta}$=11.4 Hz, $J_{1\alpha,10\beta}$=13.0 Hz, H-1α), 1.18 (3H, s, Me-17), 0.83 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (s, 2-OCOCH_3), 169.3 (s, C-18), 143.3 (d, C-15), 139.0 (d, C-16), 128.2 (s, C-13), 108.7 (d, C-14), 96.6 (s, C-4), 83.1 (s, C-8), 79.7 (t, C-3′), 73.8 (d, C-2), 69.9 (d, C-12), 68.5 (t, C-19), 47.1 (s, C-9), 45.6 (s, C-5), 44.5 (t, C-11), 41.0 (d, C-3), 39.6 (d, C-10), 29.7 (t, C-7), 27.143 (2d, C-1 and C-6), 26.9 (q, C-17), 21.1 (q, 2-OCOCH_3), 17.2 (q, C-20); EIMS *m*/*z* 428 $[M]^+$ (0.2), 413 (3), 400 (4), 385 (33), 368 (4), 358 (8), 340 (3), 325 (3), 309 (5), 269 (10), 229 (16), 164 (31), 121 (100), 105 (26), 95 (42), 91 (39), 81 (45), 55 (19). Anal.: C 64.52%, H 6.71%, N 6.41%; calcd for $C_{23}H_{28}N_2O_6$: C 64.47%, H 6.59%, N 6.54%.

4.8. Benzoylation of compound 9 to give compound 22

Benzoyl chloride (120 μL, 145 mg, 1.03 mmol) was added dropwise via syringe, at 0 °C under Ar, to a solution of 40 mg (0.103 mmol) of **9** and a little crystal of 4-DMAP in 750 μL of dry 2:1 CH_2Cl_2–pyridine. The solution was warmed at room temperature and left to stir for 24 h. Afterward, the solution was carefully poured into a crushed-ice/$NaHCO_3$ mixture (5 mL). Extraction with EtOAc (4×5 mL) was followed by removal of residual pyridine by washing with 6 N HCl and subsequent neutralization of the combined organic phase with an aqueous saturated solution of $NaHCO_3$. Finally, the organic phase was dried (Na_2SO_4) and the solvent was removed by distillation in vacuo, giving a crude of reaction that was purified by FC (39:1 CH_2Cl_2–acetone as eluent) to give pure **22** (25 mg, 50%). Amorphous, white solid; $[\alpha]_D^{20}$ −37.1 (*c* 0.197, $CHCl_3$); R_f=0.35 (silica gel, 95:5 CH_2Cl_2–acetone); IR (KBr) ν_{max} 3066, 2974, 2928, 1780, 1719, 1602, 1451, 1376, 1272, 1176, 1153, 1116, 1026, 909, 874, 716, 600 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 (2H, m, H-15, and H-16), 6.34 (1H, t, $J_{14,15}$=$J_{14,16}$=1.5 Hz, H-14), 5.01 (1H, d, $J_{3'\beta,3'\alpha}$=17.4 Hz, H-3′β), 5.01 (1H, dd, $J_{12\beta,11\alpha}$=9.9 Hz, $J_{12\beta,11\beta}$=7.1 Hz, H-12β), 4.54 (1H, d, $J_{19a,19b}$=9.4 Hz, pro-*R* H-19a), 4.42 (1H, ddd, $J_{2\beta,1\alpha}$=11.3 Hz, $J_{2\beta,1\beta}$=4.3 Hz, $J_{2\beta,3\alpha}$=10.3 Hz, H-2β), 4.39 (1H, dd, $J_{19b,6\beta}$=1.9 Hz, $J_{19b,19a}$=9.4 Hz, pro-*S* H-19b), 4.36 (1H, dd, $J_{3'\alpha,3\alpha}$=6.1 Hz, $J_{3'\alpha,3'\beta}$=17.4 Hz, H-3′α), 2.81 (1H, dddd, $J_{6\beta,6\alpha}$=14.3 Hz, $J_{6\beta,7\alpha}$=13.3 Hz, $J_{6\beta,7\beta}$=3.9 Hz, $J_{6\beta,19b}$=1.9 Hz, H-6β), 2.51 (1H, dd, $J_{3\alpha,2\beta}$=10.3 Hz, $J_{3\alpha,3'\alpha}$=6.1 Hz, H-3α), 2.31 (1H, dd, $J_{11\beta,11\alpha}$=13.2 Hz, $J_{11\beta,12\beta}$=7.1 Hz, H-11β), 2.17 (2H, m, H-6α and H-7β), 2.05 (1H, ddd, $J_{1\beta,1\alpha}$=12.8 Hz, $J_{1\beta,2\beta}$=4.3 Hz, $J_{1\beta,10\beta}$=2.1 Hz, H-1β), 1.86 (1H, dd, $J_{11\alpha,11\beta}$=13.2 Hz, $J_{11\alpha,12\beta}$=9.9 Hz, H-11α), 1.81 (1H, dd, $J_{10\beta,1\alpha}$=12.9 Hz, $J_{10\beta,1\beta}$=2.1 Hz, H-10β), 1.78 (1H, ddd, $J_{7\alpha,6\alpha}$=3.9 Hz, $J_{7\alpha,6\beta}$=13.3 Hz, $J_{7\alpha,7\beta}$=15.0 Hz, H-7α), 1.56 (1H, ddd, $J_{1\alpha,1\beta}$=12.8 Hz, $J_{1\alpha,2\beta}$=11.3 Hz, $J_{1\alpha,10\beta}$=12.9 Hz, H-1α), 1.20 (3H, s, Me-17), 0.85 (3H, s, Me-20), 2α-benzoate 8.02 (2H, dd, *J*=8.3, 1.5 Hz, H-2″ and H-6″), 7.61 (1H, tt, *J*=8.0, 1.5 Hz, H-4″), 7.47 (2H, br t, *J*=8.2 Hz, H-3″ and H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4 (s, C-18), 143.4 (d, C-15), 139.0 (d, C-16), 128.3 (s, C-13), 108.7 (d, C-14), 96.7 (s, C-4), 83.2 (s, C-8), 79.7 (t, C-3′), 74.4 (d, C-2), 70.0 (d, C-12), 68.5 (t, C-19), 47.2 (s, C-9), 45.7 (s, C-5), 44.6 (t, C-11), 41.2 (d, C-3), 39.8 (d, C-10), 29.7 (t, C-7), 27.3 (t, C-1), 27.2 (t, C-6), 26.9 (q, C-17), 17.2 (q, C-20), 2-benzoate 165.9 (s, C-7), 133.7 (d, C-4″), 129.6 (2d, C-2″ and C-6″), 129.3 (s, C-1″), 128.6 (2d, C-3″ and C-5″); EIMS *m*/*z* 490 $[M]^+$ (1), 475 (1), 462 (3), 447 (5), 368 (2), 164 (8), 121 (21), 105 (100), 95 (10), 91 (9), 77 (32). Anal.: C 68.38%, H 6.41%, N 5.59%; calcd for $C_{28}H_{30}N_2O_6$: C 68.56%, H 6.16%, N 5.71%.

4.9. Preparation of compound 23 from compound 13

A solution of **13** (60 mg, 0.167 mmol), 1,1′-thiocarbonyldiimidazole (100 mg, 0.561 mmol), and 4-DMAP (4 mg, 0.033 mmol) in dry CH_2Cl_2 (7 mL) was refluxed under Ar for 2 h. Then, the solvent was distilled in vacuo and the solid residue was purified by FC (3:2 petroleum ether–EtOAc as eluent) to give pure **23** (62 mg, 80%). Vitreous, yellowish solid; $[\alpha]_D^{20}$ −9.5 (*c* 0.042, $CHCl_3$); R_f=0.35 (silica gel, 3:2 petroleum ether–EtOAc); IR (KBr) ν_{max} 3127, 3031, 2916, 1776, 1632, 1527, 1503, 1444, 1295, 1155, 1117, 1062, 1024, 964, 873, 794, 746, 664, 603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (1H, dd, $J_{16,14}$=0.9 Hz, $J_{16,15}$=1.9 Hz, H-16), 7.43 (1H, t, $J_{15,14}$=$J_{15,16}$=1.9 Hz, H-15), 6.44 (1H, dd, $J_{14,15}$=1.9 Hz, $J_{14,16}$=0.9 Hz, H-14), 6.00 (1H, ddt, $J_{2,1\alpha}$=$J_{2,4\beta}$=2.4 Hz, $J_{2,1\beta}$=5.0 Hz, $J_{2,3}$=10.1 Hz, H-2), 5.60 (1H, dddd, $J_{3,1\alpha}$=3.0 Hz, $J_{3,1\beta}$=1.3 Hz, $J_{3,2}$=10.1 Hz, $J_{3,4\beta}$=3.4 Hz, H-3), 5.56 (1H, d, $J_{12,11\beta}$=1.2 Hz, H-12), 4.66 (1H, d, $J_{11\beta,12}$=1.2 Hz, H-11β), 4.20 (1H, dd, $J_{19a,6\beta}$=1.6 Hz, $J_{19a,19b}$=9.0 Hz, pro-*S* H-19a), 4.14 (1H, d, $J_{19b,19a}$=9.0 Hz, pro-*R* H-19b), 2.71 (1H, ddt, $J_{4\beta,1\alpha}$=2.8 Hz, $J_{4\beta,1\beta}$=$J_{4\beta,2}$=2.4 Hz, $J_{4\beta,3}$=3.4 Hz, H-4β), 2.18 (1H, m, H-1α), 2.14 (1H, m, H-1β), 1.98 (1H, m, H-7β), 1.94 (1H, br dd, $J_{8\alpha,7\alpha}$=5.2 Hz, $J_{8\alpha,7\beta}$=2.1 Hz, H-8α), 1.75 (1H, br dt, $J_{6\alpha,6\beta}$=14.0 Hz,

$J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=3.2 Hz, H-6α), 1.69 (1H, dd, $J_{10\beta,1\alpha}$=11.8 Hz, $J_{10\beta,1\beta}$=5.0 Hz, H-10β), 1.59 (1H, dddd, $J_{7\alpha,6\alpha}$=3.2 Hz, $J_{7\alpha,6\beta}$=13.5 Hz, $J_{7\alpha,7\beta}$=14.4 Hz, $J_{7\alpha,8\alpha}$=5.2 Hz, H-7α), 1.75 (1H, br dt, $J_{6\alpha,6\beta}$=14.0 Hz, $J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=3.2 Hz, H-6α), 1.23 (3H, s, Me-20), 1.18 (1H, dddd, $J_{6\beta,6\alpha}$=14.0 Hz, $J_{6\beta,7\alpha}$=13.5 Hz, $J_{6\beta,7\beta}$=3.9 Hz, $J_{6\beta,19a}$=1.6 Hz, H-6β), 1*H*-imidazole-1-carbothioate 7.69 (1H, s, H-2′), 7.10 (2H, s, H-4′ and H-5′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4 (s, C-17), 175.1 (s, C-18), 144.5 (d, C-15), 142.0 (d, C-16), 128.9 (d, C-2), 121.0 (d, C-3), 117.6 (s, C-13), 110.7 (d, C-14), 85.6 (d, C-11), 68.5 (t, C-19), 52.2 (d, C-4), 47.3 (d, C-12), 46.1 (s, C-9), 44.1 (d, C-8), 41.3 (s, C-5), 41.1 (d, C-10), 31.0 (t, C-6), 22.8 (t, C-1), 16.9 (q, C-20), 16.6 (t, C-7), 1*H*-imidazole-1-carbothioate 223.4 (s, C=S), 134.9 (d, C-2′), 132.0 (d, C-4′), 121.9 (d, C-5′); EIMS *m*/*z* $[M]^+$ absent, 453 $[M-CH_3]^+$ (1), 421 (2), 374 (29), 372 (25), 356 (6), 340 (80), 261 (72), 203 (22), 157 (30), 145 (39), 129 (33), 113 (84), 108 (86), 91 (100), 81 (43), 76 (53), 65 (17), 53 (20). Anal.: C 61.28%, H 5.02%, N 6.03%, S 6.91%; calcd for $C_{24}H_{24}N_2O_6S$: C 61.52%, H 5.16%, N 5.98%, S 6.84%.

4.10. Preparation of compound 24 from compound 23

A solution of **23** (50 mg, 0.107 mmol), 150 μL (162 mg, 0.535 mmol) of 96% *n*-Bu_3SnH, and 2,2′-azo-bis-isobutyronitrile (AIBN, 3 mg, 0.018 mmol) in toluene (5 mL) was heated at reflux under Ar for 6 h. Then, the mixture was evaporated to a little volume and partitioned between acetonitrile and *n*-hexane (5 mL each). The acetonitrile layer was washed with *n*-hexane (4×5 mL) to remove the residual tin compounds. The acetonitrile phase was distilled in vacuo and the residue was purified by FC (39:1 acetone–CH_2Cl_2 as eluent) to give **24** (16 mg, 45%). Colorless rectangular plaques (EtOAc–*n*-pentane); mp 211–214 °C; $[\alpha]_D^{20}$ −91.3 (*c* 0.196, $CHCl_3$); R_f=0.50 (silica gel, 3:2 petroleum ether–EtOAc); IR (KBr) ν_{max} 3142, 3107, 2963, 2860, 1780, 1766, 1502, 1450, 1352, 1296, 1176, 1152, 1035, 966, 873, 785, 702, 602 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (1H, t, $J_{15,14}$=$J_{15,16}$=1.8 Hz, H-15), 7.31 (1H, ddd, $J_{16,12b}$=1.0 Hz, $J_{16,14}$=0.8 Hz, $J_{16,15}$=1.8 Hz, H-16), 6.34 (1H, dd, $J_{14,15}$=1.8 Hz, $J_{14,16}$=0.8 Hz, H-14), 5.97 (1H, ddt, $J_{2,1\alpha}$=$J_{2,4\beta}$=2.4 Hz, $J_{2,1\beta}$=5.0 Hz, $J_{2,3}$=10.0 Hz, H-2), 5.60 (1H, dddd, $J_{3,1\alpha}$=3.1 Hz, $J_{3,1\beta}$=1.3 Hz, $J_{3,2}$=10.0 Hz, $J_{3,4\beta}$=3.4 Hz, H-3), 4.35 (1H, dd, $J_{11\beta,12a}$=4.0 Hz, $J_{11\beta,12b}$=9.2 Hz, H-11β), 4.19 (2H, s, H-19a and H-19b), 2.74 (1H, tt, $J_{4\beta,1\alpha}$=$J_{4\beta,3}$=3.4 Hz, $J_{4\beta,1\beta}$=$J_{4\beta,2}$=2.4 Hz, H-4β), 2.71 (1H, br dd, $J_{12a,11\beta}$=4.0 Hz, $J_{12a,12b}$=15.2 Hz, H-12a), 2.61 (1H, ddd, $J_{12b,11\beta}$=9.2 Hz, $J_{12b,12a}$=15.2 Hz, $J_{12b,16}$=1.0 Hz, H-12b), 2.54 (1H, ddd, $J_{8\alpha,6\alpha}$=1.2 Hz, $J_{8\alpha,7\alpha}$=5.1 Hz, $J_{8\alpha,7\beta}$=2.3 Hz, H-8α), 2.16 (1H, dddd, $J_{7\beta,6\alpha}$=3.8 Hz, $J_{7\beta,6\beta}$=4.0 Hz, $J_{7\beta,7\alpha}$=14.8 Hz, $J_{7\beta,8\alpha}$=2.3 Hz, H-7β), 2.09 (1H, m, H-1β), 2.05 (1H, m, H-1α), 1.82 (1H, m, H-6α), 1.79 (1H, dd, $J_{10\beta,1\alpha}$=11.1 Hz, $J_{10\beta,1\beta}$=5.3 Hz, H-10β), 1.77 (1H, dddd, $J_{7\alpha,6\alpha}$=4.1 Hz, $J_{7\alpha,6\beta}$=13.6 Hz, $J_{7\alpha,7\beta}$=14.8 Hz, $J_{7\alpha,8\alpha}$=5.1 Hz, H-7α), 1.27 (1H, ddd, $J_{6\beta,6\alpha}$=14.2 Hz, $J_{6\beta,7\alpha}$=13.6 Hz, $J_{6\beta,7\beta}$=4.0 Hz, H-6β), 1.03 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2 (s, C-17), 175.3 (s, C-18), 143.2 (d, C-15), 140.2 (d, C-16), 128.8 (d, C-2), 121.1 (d, C-3), 119.7 (s, C-13), 111.3 (d, C-14), 83.8 (d, C-11), 68.7 (t, C-19), 52.1 (d, C-4), 45.6 (d, C-8), 42.9 (s, C-9), 41.0 (s, C-5), 39.8 (d, C-10), 31.2 (t, C-6), 25.9 (t, C-12), 22.7 (t, C-1), 17.2 (q, C-20), 16.7 (t, C-7); EIMS *m*/*z* 342 $[M]^+$ (56), 327 (1), 261 (100), 233 (7), 215 (7), 203 (8), 157 (24), 145 (24), 117 (28), 105 (27), 91 (57), 81 (34), 53 (17). Anal.: C 70.51%, H 6.31%; calcd for $C_{20}H_{22}O_5$: C 70.16%, H 6.48%.

4.11. Biological assays

As described,[22] the recombinant CHO cells (hMOP-CHO, hDOP-CHO, and hKOP-CHO) were produced by stable transfection with the respective human opioid receptor cDNA, and provided by Dr. Larry Toll (SRI International, CA). The cells were grown on plastic flasks in DMEM (90%) (hDOP-CHO and hKOP-CHO) or DMEM/F-12 (45%/45%) medium (hMOP-CHO) containing 10% FetalClone II (HyClone) and Geneticin (G-418: 0.10–0.2 mg/mL) (Invitrogen) under 95% air/5% CO_2 at 37 °C. Cell monolayers were harvested and frozen in −80 °C.

4.11.1. Opioid binding assays

We used either [^{125}I]IOXY (6β-iodo-3,14-dihydroxy-17-cyclopropylmethyl-4,5α-epoxymorphinan) (SA=2200 Ci/mmol) to label μ, δ and κ binding sites [20] or [^{3}H][D-Ala^2-MePhe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO, SA=44–48 Ci/mmol) to label MOP, [^{3}H][D-Ala^2,D-Leu^5]enkephalin ([^{3}H]DADLE, SA=40–50 Ci/mmol) to label DOP and [^{3}H](−)-U69,593 (SA=50 Ci/mmol) to label KOP binding sites. On the day of the assay, cell pellets were thawed on ice for 15 min then homogenized with a polytron in 10 mL/pellet of ice-cold 10 mM Tris–HCl, pH 7.4. Membranes were then centrifuged at 30,000×*g* for 10 min, resuspended in 10 mL/pellet ice-cold 10 mM Tris–HCl, pH 7.4 and again centrifuged 30,000×*g* for 10 min. Membranes were then resuspended in 25 °C 50 mM Tris–HCl, pH 7.4 (~100 mL/pellet hMOP-CHO, 50 mL/pellet hDOP-CHO, and 120 mL/pellet hKOP-CHO). All assays took place in 50 mM Tris–HCl, pH 7.4, with a protease inhibitor cocktail [bacitracin (100 μg/mL), bestatin (10 μg/mL), leupeptin (4 μg/mL), and chymostatin (2 μg/mL)], in a final assay volume of 1.0 mL. All drug dilution curves were made up with buffer containing 1 mg/mL BSA. Non-specific binding was determined using 10 μM naloxone (for [^{125}I]IOXY binding) or 20 μM levallorphan ([^{3}H]DAMGO and [^{3}H]DADLE) or 10 μM (−)-U69,593 (for [^{3}H]U69,593 binding). [^{125}I]IOXY concentrations were ~10 pM. The other [^{3}H]radioligands were used at ~2 nM concentrations. Triplicate samples were filtered with Brandell Cell Harvesters (Biomedical Research & Development Inc., Gaithersburg, MD), over Whatman GF/B filters, after a 2 h incubation at 25 °C. For [^{125}I]IOXY binding, the filters were punched into 12×75 mm glass test tubes and counted in a Micromedic gamma counter at 80% efficiency. For [^{3}H]DAMGO, [^{3}H]DADLE, and [^{3}H]-(−)U69,593 binding, the filters were punched into 24-well plates to which was added 0.6 mL of LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 44% efficiency. Opioid binding assays had ~30 μg protein per assay tube. Inhibition curves were generated by displacing a single concentration of radioligand by 10 concentrations of drug.

4.11.2. [^{35}S]GTP-γ-S binding assays

The [^{35}S]-GTP-γ-S assays were conducted as described elsewhere.[22] In this description, buffer 'A' is 50 mM Tris–HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA and buffer 'B' is buffer A plus 1.67 mM DTT and 0.15% BSA. On the day of the assay, cells were thawed on ice for 15 min and homogenized using a polytron in 50 mM Tris–HCl, pH 7.4, containing 4 μg/mL leupeptin, 2 μg/mL chymostatin, 10 μg/mL bestatin, and 100 μg/mL bacitracin. The homogenate was centrifuged at 30,000×*g* for 10 min at 4 °C, and the supernatant discarded. The membrane pellets were resuspended in buffer B and used for [^{35}S]GTP-γ-S binding assays. [^{35}S]GTP-γ-S binding was determined as described previously. Briefly, test tubes received the following additions: 50 μL buffer A plus 0.1% BSA, 50 μL GDP in buffer A/0.1% BSA (final concentration=40 μM), 50 μL drug in buffer A/0.1% BSA, 50 μL [^{35}S]GTP-γ-S in buffer A/0.1% BSA (final concentration=50 pM), and 300 μL of cell membranes (50 μg of protein) in buffer B. The final concentrations of reagents in the [^{35}S]GTP-γ-S binding assays were 50 mM Tris–HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 40 μM GDP, and 0.1% BSA. Incubations proceeded for 3 h at 25 °C. Nonspecific binding was determined using GTP-γ-S (40 μM). Bound and free [^{35}S]-GTP-γ-S were separated by vacuum filtration through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

4.11.3. Data analysis and statistics

These methods are described elsewhere.[22–24] For the [^{35}S]GTP-γ-S binding experiments, the percent stimulation of [^{35}S]GTP-γ-S binding was calculated according to the following formula: $(S-B)/B\times100$, where B is the basal level of [^{35}S]GTP-γ-S binding and S is the stimulated level of [^{35}S]GTP-γ-S binding. Agonist dose–response curves (10 points/curve) are generated, and the data of several experiments, typically 3, are pooled. The EC_{50} values (the concentration that produces 50% maximal stimulation of [^{35}S]GTP-γ-S binding) and E_{max} are determined using either the program MLAB-PC (Civilized Software, Bethesda, MD), KaleidaGraph (Version 3.6.4, Synergy Software, Reading, PA) or Prism 4.0 (GraphPad Software, Inc., San Diego, CA). In most cases, the percent stimulation is reported as a percent of the maximal stimulation as determined with 1000 nM DAMGO, 500 nM SNC80 or 500 nM (−)-U50,488 in the appropriate cell type. For determination of K_e values using the 'shift' experimental design, agonist (DAMGO, (−)-U50,488 or SNC80) dose–response curves are generated, using the appropriate cell type, in the absence and presence (10 points/curve) of a test agent. The data of several experiments, typically 3, are pooled, and the K_e values were calculated according to the equation: [Test Drug]/($EC_{50\text{-}2}/EC_{50\text{-}1}-1$), where $EC_{50\text{-}2}$ is the EC_{50} value in the presence of the test drug and $EC_{50\text{-}1}$ is the value in the absence of the test drug. For opioid binding experiments, the pooled data of three experiments (typically 30 data points) were fit to the two-parameter logistic equation for the best-fit estimates of the IC_{50} and N values: $Y=100/(1+([\text{INHIBITOR}]/IC_{50})^N)$, where 'Y' is the percent of control value. K_i values for test drugs are calculated according to the standard equation: $K_i=IC_{50}/(1+[\text{radioligand}]/K_d)$. For the [^{125}I]IOXY experiments, the concentration of [^{125}I]IOXY was far below its K_d values for MOP, KOP or DOP, so the IC_{50} values equaled the K_i values. For the [^{3}H]radioligands, the following K_d values (nM±SD, $n=3$) were used in the K_i calculation: [^{3}H]DAMGO (0.93±0.04), [^{3}H]DADLE (1.9±0.3), and [^{3}H](−)-U69,593 (11±0.6). The corresponding B_{max} values were (fmol/mg protein±SD, $n=3$) [^{3}H]DAMGO (1912±68), [^{3}H]DADLE (3655±391), and [^{3}H](−)-U69,593 (3320±364).

4.11.4. Drugs

Salvinorin A related compounds were made up in 100% DMSO+14.3 M 2-mercaptoethanol to produce a 10 mM solution. Compounds were then aliquoted into 50 μL volumes in microfuge tubes and frozen at −80° C until the day of the assay. Any leftover compound was discarded.

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16. Since all the reported compounds (**1**–**24**) belong to the *enantio* series[1,9–13] the α- or β-configuration should be described as *ent*-β or *ent*-α, indicating that the substituent is placed, respectively, below or above the plane of the formulas. However, for clarity we use throughout the text the α- or β-nomenclature, indicating that a substituent is placed, respectively, below or above the plane of the formulas depicted for the described substances.
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19. It is of interest to note that the β-oriented pyrazoline ring of **19** and **20** causes a strong shielding effect on the H-2β proton, which resonates at δ 0.33 and 0.40, respectively. Moreover, only one (H-3′α) of the 3′-methylene protons of the pyrazoline part of **9** and **19**–**22** is coupled with the vicinal H-3α proton, because in these compounds, and in other 3β,4β-pyrazoline derivatives of neoclerodan-18,19-olides, the dihedral angle between H-3′β and H-3α is close to 90° (see Rodríguez, B. *Magn. Reson. Chem.* **2001**, *39*, 150–154).
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