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Design and Synthesis of A-Ring Simplified Pyripyropene A Analogs as Potent and Selective Synthetic SOAT2 Inhibitors

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Abstract: Currently, pyripyropene A, which is isolated from the culture broth of *Aspergillus fumigatus* FO-1289, is the only compound known to strongly and selectively inhibit the isozyme sterol *O*-acyltransferase 2 (SOAT2). To aid in the development of new cholesterol-lowering or antiatherosclerotic agents, new A-ring simplified pyripyropene A analogs were designed and synthesized based on the total synthesis and the results of the structure–activity relationship studies of pyripyropene A achieved by our group. Among the analogs, two A-ring simplified pyripyropene A analogs exhibited equally efficient SOAT2 inhibitory activity compared with that of natural pyripyropene A. These new analogs are the most potent and selective SOAT2 inhibitors to be used as synthetic compounds and attractive seed compounds for the development of drug for dyslipidemia, including atherosclerotic disease and steatosis.

Introduction

Sterol *O*-acyltransferase (SOAT), which is also known as acyl-CoA: cholesterol acyltransferase (ACAT)[1] plays an important role in the metabolism of cholesterol in mammals. Hence, a large number of synthetic SOAT inhibitors, such as ureas, imidazoles, and amides, have been reported for the treatment of hypercholesterolemia and atherosclerosis.[2] Among these inhibitors, the development of avasimibe[3] and pactimibe[4] had been expected; however, their clinical studies were unsuccessful. To the best of our knowledge, thus far, no new cholesterol-lowering or antiatherosclerotic agents from SOAT inhibitors have emerged in the market.[5]

During the course of our screening for naturally derived SOAT inhibitors with a chemical structure different from the synthetic inhibitors developed in the 1990s, a number of new natural products have been discovered.[6] Among these natural products, the fungal metabolite pyripyropene A (PPPA, **1**) has been reported to be one of the most potent SOAT inhibitors in an enzyme assay using rat liver microsomes (Figure 1).[7] Accordingly, approximately 200 derivatives have been semisynthetically prepared from **1** for a first structure–activity relationship (SAR) study. Certain derivatives have been reported to be not only more potent than **1**, but also active in decreasing the absorption of cholesterol *in vivo* from the intestines of hamsters.[8] Recent molecular biological studies have revealed the presence of two isozymes, namely, SOAT1 and SOAT2.[9] SOAT1 is ubiquitously expressed, and a high-level expression is observed in sebaceous glands, steroidogenic tissues, and macrophages, while SOAT2 is predominantly expressed in the liver (hepatocytes) and intestine.[10] Hence, selective inhibitors toward SOAT2 are expected to demonstrate potential as better drug candidates with fewer side effects as cholesterol-lowering or antiatherosclerotic agents. A cell-based assay using SOAT1- or SOAT2-expressing Chinese hamster ovary (CHO) cells has been developed by our group, and the selectivity of microbial SOAT inhibitors previously discovered by our group has been investigated.[11] Consequently, only **1** is confirmed to be a potent and selective inhibitor toward SOAT2 among reported SOAT inhibitors including our natural SOAT inhibitors. Moreover, the *in vivo* efficacy of **1** in atherosclerosis has been also demonstrated.[12] PPPA derivatives synthesized previously have been evaluated in this cell-based assay to investigate their selective inhibition toward the SOAT isozymes.[11b] Compared to **1**, several PPPA derivatives exhibited more potent SOAT2 inhibitory activity; however, unfortunately, the isozyme selectivity for all of the synthesized PPPA derivatives was considerably less than that of **1**. Therefore, new PPPA derivatives (>200)[13] are prepared in a semisynthetic manner, and the second SAR of **1** is elucidated (Figure 1). Finally, 7-*O*-*p*-cyanobenzoyl PPPA derivative **2**,[13a] 1,11-*O*-*o*-methylbenzylidene acetal derivative **3**,[13c] and 1,11-*O*-*o,o*-dimethylbenzylidene acetal derivative **4**,[13c] which exhibited SOAT2 inhibitory activity and isozyme selectivity greater than those of **1**, have been developed (Figure 2 and Table 1).

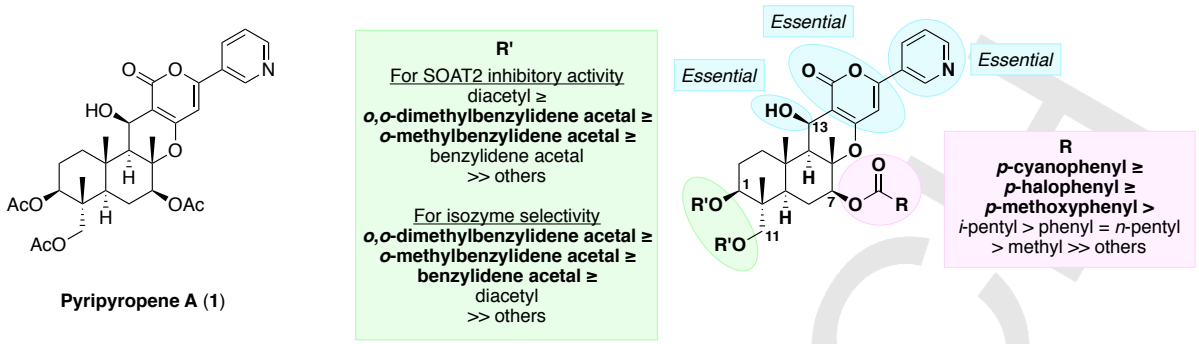


Figure 1. Structure and summary of SAR of pyripyropene A (**1**).

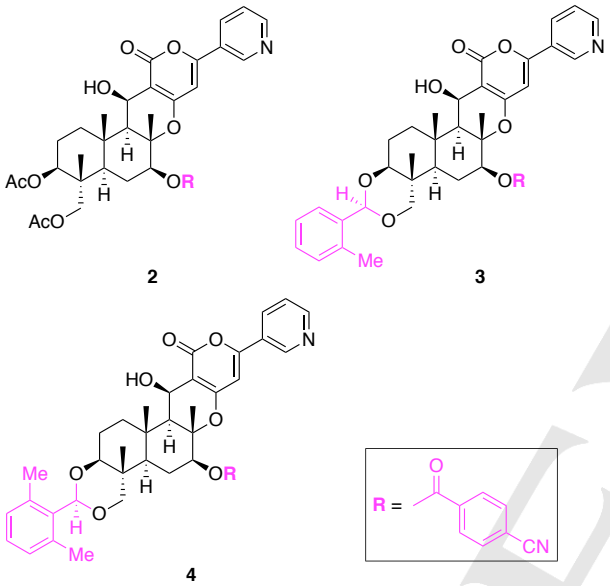


Figure 2. Synthetic PPPA derivatives **2**–**4** developed by our group as potent and selective SOAT2 inhibitors.

Table 1. SOAT1 and SOAT2 inhibitory activities and isozyme selectivities of pyripyropene A (PPPA, **1**) and synthetic PPPA derivatives **2–4**.

	IC_{50} (μM)		
Compounds	SOAT1	SOAT2	SI[a]
Pyripyropene A (**1**)	>80.00	0.0700	>1000
2	4.16	0.0009	4622
3	>72.80	0.0118	>6161
4	>71.20	0.0072	>9916

[a] Selectivity index (SI): IC_{50}(SOAT1) / IC_{50}(SOAT2)

Furthermore, compared to **1**, these PPPA derivatives, particularly **3**, exhibited more potent *in vivo* efficacy.[14]

As described above, the attractive lead compounds for the development of drugs for dyslipidemia were successfully obtained. Next, the third SAR studies of **1** were planned, where new analogs that were not derived from chemically natural PPPA, namely, A-ring simplified PPPA analogs, were focused on. Hence, a new, efficient, synthetic route for **1** and their analogs was required, and the stereocontrolled total synthesis of **1** was achieved by our group.[13d,15] Herein, we report the design, synthesis, and biological evaluation of novel A-ring simplified PPPA analogs by an extension of all our research results on PPPA (**1**), which permit the discovery of the most potent and selective SOAT2 inhibitors as synthetic compounds.[16] Recently, Li and Nan et al. have reported similar results by the application of our total synthesis, where a new analog exhibited more potent and selective SOAT2 inhibitory activity compared with that observed for **1**.[17] In addition, we report the results from the biological evaluation of the analog in our assay system.

Results and Discussion

Figure 3 shows the designed A-ring simplified PPPA analogs for the third SAR study of **1**. In our total synthesis, (*R*)-carvone, corresponding to the B-ring of **1** as the starting material, was selected, and subsequent ring construction was achieved in order of A, C, and D (by coupling with the E-ring). Hence, the exclusion of the several steps for the construction of the A-ring from the total synthesis possibly affords the designed A-ring simplified PPPA analogs **5–9**.

First, the synthesis of **5**, which is the simplest analog in this paper, was performed according to our total synthesis (Scheme 1). The regioselective hydrogenation of (*R*)-(−)-carvone[18] afforded **11**, which was subjected to β-epoxide formation. First, acetoxybromination under the conditions employed previously by our group (NBA and AgOAc)[15] was performed to smoothly afford **12a**; however, second methanolysis and epoxide formation did not afford desired product **13**, in addition to substrate decomposition. After the screening of the transformation, the bromohydrin was prepared by treatment with NBS and 1 N $HClO_4$,[19] followed by epoxide formation via the exposure to DBU, furnishing desired β-epoxide **13** in 55% yield over three steps from (*R*)-(−)-carvone. The Wittig olefination of **13**, instead of Peterson olefination employed in our total synthesis,[15] and subsequent acidic work-up led to the opening of the epoxide, furnishing the corresponding γ-hydroxy-α,β-unsaturated aldehyde in 95% yield;

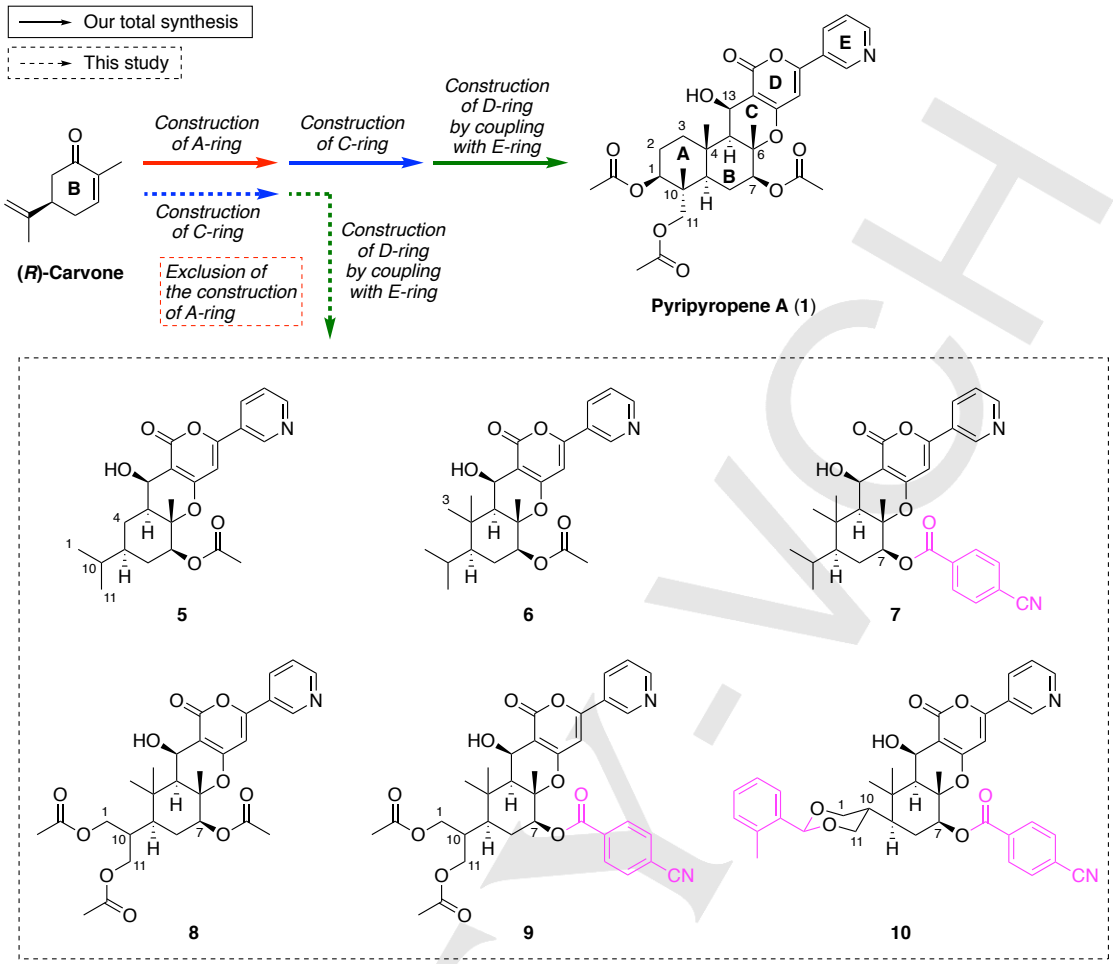
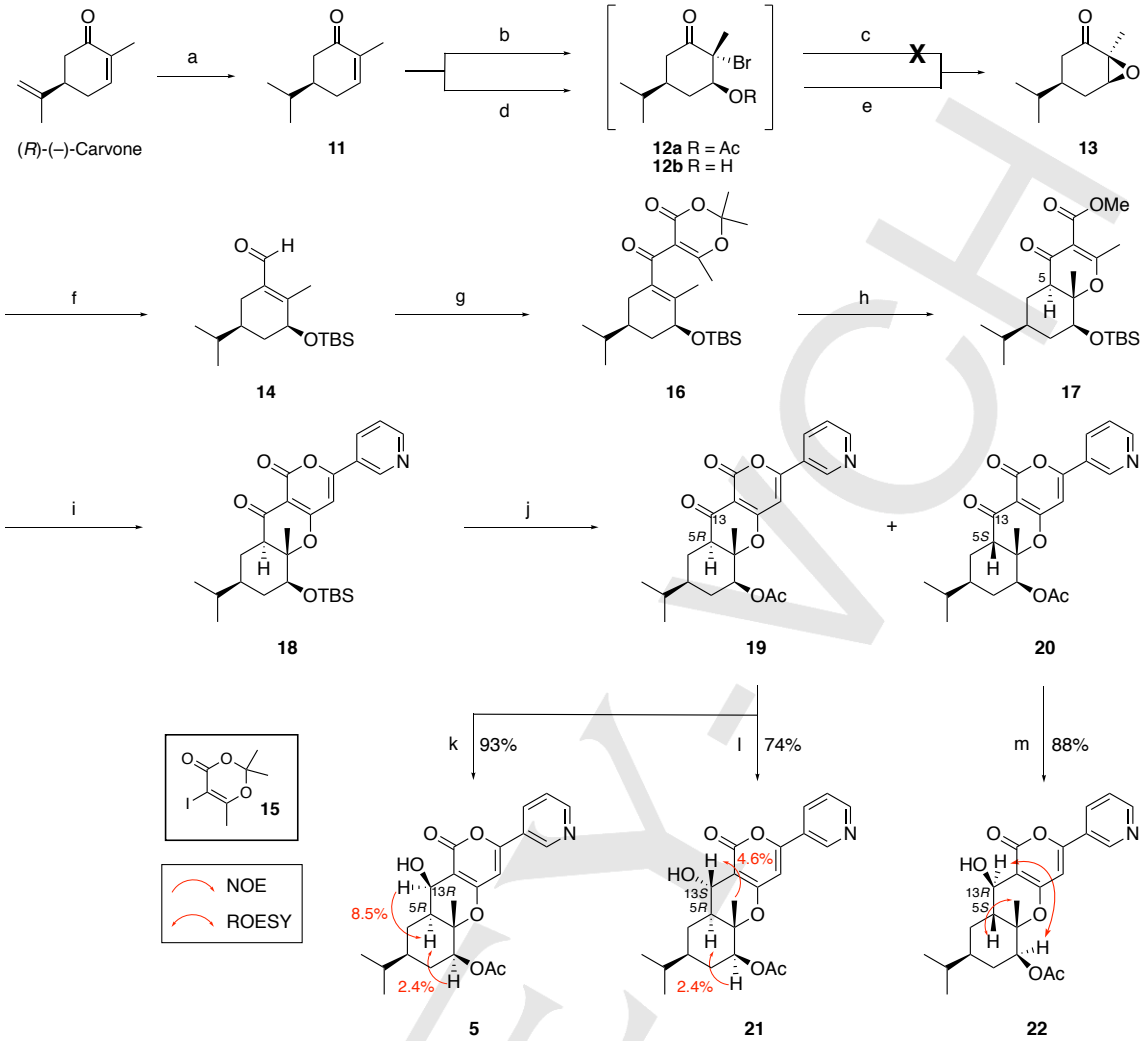


Figure 3. The developed synthetic strategy for the new target A-ring simplified PPPA analogs **5–10** for the third SAR study of PPPA (**1**).

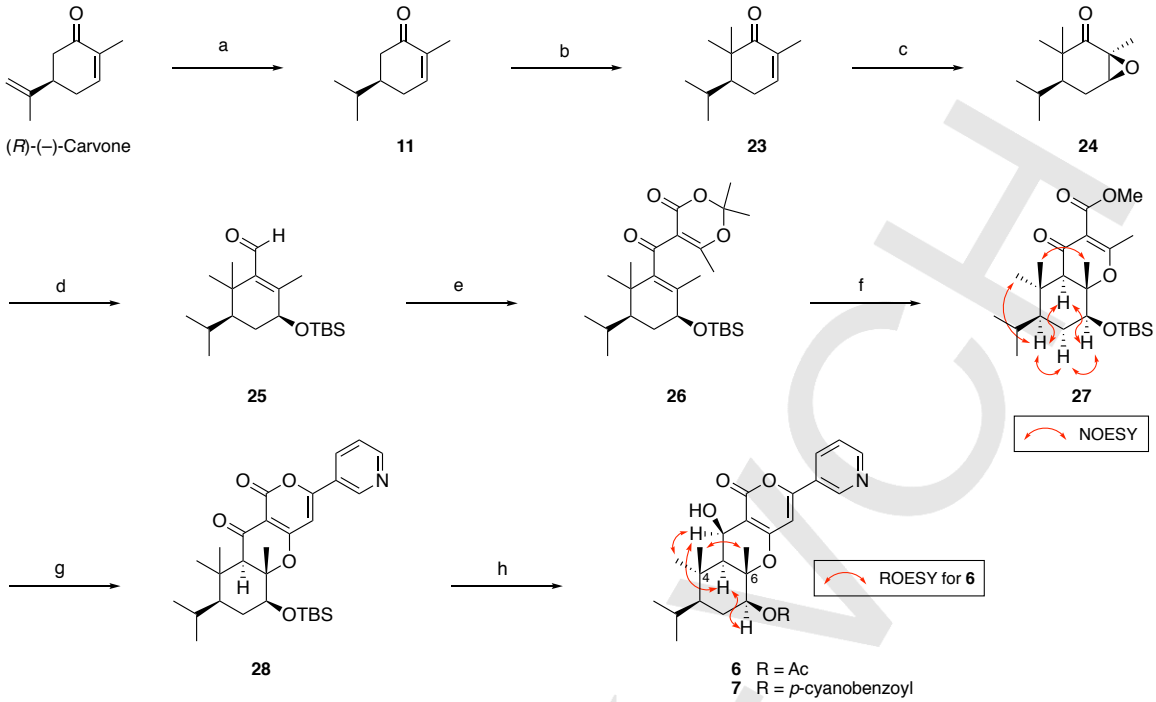
this aldehyde was protected as a TBS ether to afford aldehyde **14** in 76% yield. The treatment of **14** with a corresponding Grignard reagent derived from iodide **15**[20] followed by the Dess–Martin oxidation afforded diketo ester equivalent **16** in 68% in two steps. The methanolysis of **16** and the heating of the corresponding diketo methyl ester with DBU at 100 °C furnished an inseparable diastereomixture (3 : 1 at C5) of dihydro-γ-pyrone **17** by the intramolecular oxa-conjugate addition of the resulting conjugated enol in 80% yield over two steps.[21] The enolization of **17** by treatment with LHMDS, γ-acylation with nicotinoyl chloride, and intramolecular cyclization for the formation of γ-pyrone in one pot afforded **18** in 66% yield as an inseparable 3 : 1 diastereomixture. The deprotection of the TBS ether under acidic conditions and acetylation furnished desired ketoacetate **19** and its C5 epimer **20** in isolated yields of 53% and 14%, respectively, over 2 steps. Finally, the reduction of the keto group of **19** with $NaBH_4$ afforded interesting results; reduction with $NaBH_4$ and $NaBH_4$–$CeCl_3$ (Luche conditions[22]) afforded desired β-alcohol **5** and its C13 epimer **21** in 93% and 74% yields, respectively, the stereochemistry of which was confirmed by NOE and ROESY experiments. In addition, the reduction of **20** was attempted. The reaction with $NaBH_4$–$CeCl_3$ afforded β-alcohol **22** in 88% yield; however, the reduction using only $NaBH_4$ led to decomposition without any reduced products. The reason why stereochemistry observed with the presence or absence of $CeCl_3$ differs is still unclear; however, similar results using other substrates have been reported previously by the Krief[23] and Jean[24] groups, and the Krief group has provided a promising hypothesis for these results. During the reduction of **19** in the presence of $CeCl_3$, some $CeCl_3$-containing species would be located at the less-hindered α-face of the 13-ketone, which blocked the nucleophilic attack from the α-face. Hence, the subsequent attack of the hydride would be forced from the original hindered β-face, affording α-alcohol **21**. Conversely, the reaction of **19** in the absence of $CeCl_3$ would be reduced by the hydride from the less-hindered α-face, affording β-alcohol **5**. During the reduction of **20**, the $CeCl_3$-containing species would be located at the less-hindered β-face (convex face) of the 13-ketone of **20**, and the attack of the hydride would be forced from the original hindered α-face (concave face), affording β-alcohol **22**. The corresponding α-alcohol, which was obtained by the reduction of **20** with $NaBH_4$, possibly decomposed owing to its unstability.

Next, A-ring simplified PPPA analogs **6** and **7** were also synthesized (Scheme 2). The double methylation[25] of known ketone **11**, which was obtained by the regioselective hydrogenation of (*R*)-(−)-carvone, afforded **23**. The acetoxybromination of α,β-unsaturated ketone **23** under the conditions previously reported by our group (NBA and AgOAc)[15] smoothly proceeded to furnish the corresponding bromoacetate, which was subjected to methanolysis to afford β-epoxide **24** in 62% yield over five steps. The Peterson olefination of **24**



Scheme 1. Synthesis of A-ring simplified PPPA analogs **5**, **21**, and **22**. *Reagents and conditions*: a) PtO_2, H_2, neat, rt; b) NBA, AgOAc, AcOH, rt; c) K_2CO_3, MeOH, rt; d) NBS, 1N $HClO_4$, H_2O-dioxane, 0 °C; e) DBU, rt, 55% over three steps; f) 1. $MeOCH_2PPh_3Cl$, *t*-BuOK, THF, then aq. HCO_2H, rt, 95%, 2. TBSCl, imidazole, DMAP, DMF, 50 °C, 76%; g) 1. **15**, *i*-PrMgCl, THF, –30 °C, 2. DMP, CH_2Cl_2, 0 °C, 68% over two steps; h) 1. MeOH, toluene, 90 °C, 2. DBU, toluene, 100 °C, 80% over two steps (dr = 3:1); i) LHMDS, nicotinoyl chloride hydrochloride, THF, –78 °C to rt, 66% (dr = 3:1); j) 1. AcCl, MeOH, rt, 2. Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 53% over two steps for **19**, 14% over two steps for **20**; k) $NaBH_4$, MeOH, 0 °C, 93%; l) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, 74%; m) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, 88%.

followed by the hydrolysis and TBS protection of the resulting secondary alcohol afforded α,β-unsaturated aldehyde **25**. In this case, instead of Peterson olefination as shown in Scheme 1, the Wittig reaction gave no reaction. This result would be caused by the steric hindrance at the α-positions of the carbonyl group and is in agreement with that reported previously.[15] Diketo ester equivalent **26** was obtained in 44% yield over 4 steps by coupling between aldehyde **25** and the corresponding Grignard reagent derived from iodide **15** followed by the Dess–Martin oxidation. The methanolysis of **26** followed by treatment with DBU in toluene at 100°C stereoselectively afforded dihydro-γ-pyrone **27** by the intramolecular oxa-conjugate addition in 69% yield over 2 steps without any other stereoisomer unlike the synthesis of **19** shown in Scheme 1. The stereochemistries of **27** were confirmed by NOESY. This result is in agreement with that obtained in the total synthesis of **1** reported previously by our group.[15] The stereoselective α-protonation at C5 of the resulting enolate after the intramolecular oxa-conjugate addition can be completely controlled by two key sterically demanding groups on the β-face, originating from the C4 and C6-axial methyl groups, respectively. Compound **28** bearing the pyridyl-α-pyrone moiety was synthesized in 44% yield by the coupling of the corresponding enolate derived from **27** with nicotinoyl chloride. Finally, the deprotection of the TBS group in **28** was conducted under acidic conditions, followed by acylation (acetyl or *p*-cyanobenzoyl) and the stereoselective Luche reduction to furnish A-ring simplified PPPA analogs **6** and **7** in 73% and 44%, respectively, over three steps. Notably, the final Luche reduction in Scheme 2 and the total synthesis of **1** reported previously by our group exhibited the same stereoselectivity, but that of the Luche reduction of **19** in Scheme 1 was reversed. In the former case, the presence of the two axial methyl groups at C4 and C6 would completely block the reduction from the extremely hindered β-face, leading to the sole formation of the β-hydroxy group, which was reduced from the less-hindered α-face. The latter would lead to the opposite stereoselectivity in the Luche reduction because of the decrease in the steric hindrance by the absence of the C4-axial methyl group.



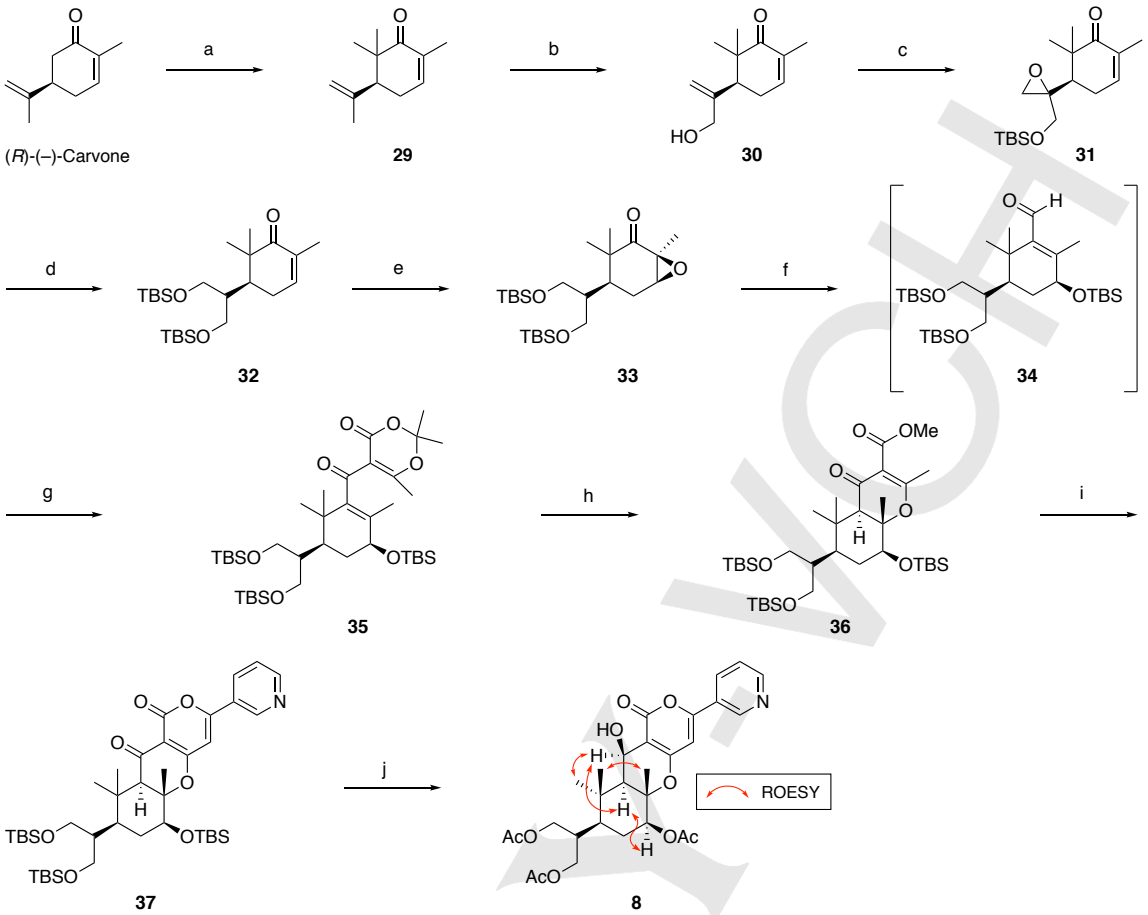
Scheme 2. Synthesis of the A-ring simplified PPPA analogs **6** and **7**. *Reagents and conditions*: a) PtO_2, H_2, neat, rt; b) 1. LDA, MeI, THF, 0 °C to rt, 2. LDA, MeI, THF, 0 °C to rt; c) NBA, AgOAc, AcOH, rt, 2. K_2CO_3, MeOH, rt, 62% over five steps; d) 1) $TMSCH_2OMe$, *s*-BuLi, *t*-BuOK, THF, –78 °C to rt, 2. TBSCl, imidazole, DMAP, DMF, 60 °C; e) 1. **15**, *i*-PrMgCl, THF, –30 °C to rt, 2. DMP, CH_2Cl_2, rt, 44% over four steps; f) 1. MeOH, toluene, 90 °C, 2. DBU, toluene, 100 °C, 69% over two steps; g) LHMDS, nicotinoyl chloride hydrochloride, THF, –78 °C to rt, 44%; h) 1. AcCl, MeOH, THF, 0 °C, 2. Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt or *p*-cyanobenzoic acid, EDCI, DMAP, CH_2Cl_2, rt, 3. $NaBH_4$, $CeCl_3$, MeOH, –78 °C to 0 °C, 73% over three steps for **6**, 44% over three steps for **7**.

Finally, A-ring simplified PPPA analogs **8**, **9**, and **10** were synthesized (Schemes 3 and 4). First, **8** was synthesized starting with the chlorination[26] of known 6,6-dimethylcarvone (**29**).[25] The hydrolysis[27] of the resulting allyl chloride afforded allyl alcohol **30** in 92% yield (Scheme 3). Next, the regioselective hydroboration followed by oxidation[28] of **30** was performed under various conditions; however, either a reaction did not proceed or substrate decomposition was observed. Hence, the titanocene-catalyzed reductive epoxide-opening reaction[28,29] by the TBS protection of a hydroxy group afforded **31** as a diastereomixture (ca. 3 : 2) in high yield over 2 steps. The reductive epoxide-opening reaction in the presence of water as the hydride source[30] of **31** afforded the desired primary alcohol in 72% yield, which was protected as a TBS ether to afford **32** in 93% yield. The subsequent conversion of **32** to **8** was carried out according to our total synthesis.[15] The acetoxybromination of **32** and methanolysis furnished β-epoxide **33** in 60% yield over two steps, which was subjected to the Peterson olefination, TBS protection, the coupling of the resulting aldehyde **34** with **15**, and the Dess–Martin oxidation, furnishing **35** in 42% yield over 4 steps. The methanolysis of **35** and stereoselective cyclization afforded dihydro-γ-pyrone **36** in 66% yield over 2 steps. The subsequent construction of the pyridyl-γ-pyrone moiety afforded **37** in 34% yield. Finally, the deprotection of the TBS ether, acetylation, and Luche reduction afforded A-ring simplified PPPA analog **8** in 51% yield over 3 steps.

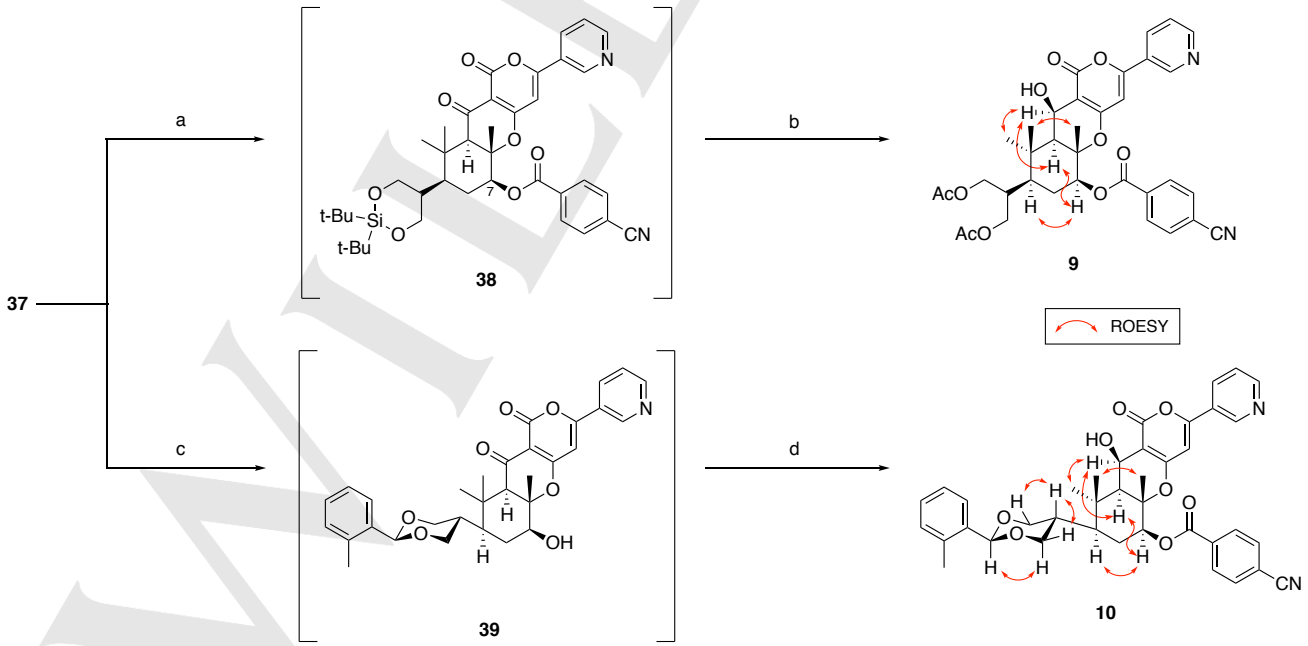
A-ring simplified PPPA analogs **9** and **10** were derived from **37** (Scheme 4). Desilylation under acidic conditions, silylene acetal formation of 1,3-diol in the resulting triol, and *p*-cyanobenzoylation at C7 afforded **38**, which was converted into the desired **9** by desilylation, acetylation, and the Luche reduction in a total yield of 38% over 6 steps from **37**. In addition, another analog **10** was similarly synthesized from **37** (total yield of 25% over 4 steps) by desilylation, acetalization with *o*-tolualdehyde, *p*-cyanobenzoylation of C7 hydroxy group of the resulting acetal **39**, and the Luche reduction.

New A-ring simplified PPPA analogs **5**–**10**, **21**, and **22** were synthesized according to our total synthesis.[15] Moreover, A-ring simplified PPPA analog **40**, which exhibited more potent and selective SOAT2 inhibitory activity compared with that of **1**, was synthesized according to the report by Li and Nan et al.[17,21] (Figure 4). Table 2 summarizes the SOAT inhibitory activities and isozyme selectivities of all compounds.

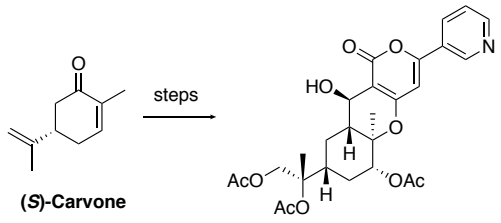
The SOAT2 inhibitory activities and isozyme selectivities of **5**, **8**, **21**, and **22** disappeared, while those of **6** considerably decreased in comparison with those of **1**. These new analogs lack A-ring. Therefore, pyripyropene A (**1**), bearing a rigid steroid-like skeleton, exhibits a potent inhibitory activity because the three acetoxy groups are located in the suitable position when binding to SOAT2.[31] In contrast, among analogs **7**, **9**, and **10**, bearing the *p*-cyanobenzoyl group, analogs **7** and **10** exhibited the same SOAT2 inhibitory activity compared with that of **1**; however, that of analog **9** slightly decreased. These new analogs also lack A-ring; however, since they bear the *p*-cyanobenzoyl group at C7 (and the *o*-methylbenzylideneacetal at C1 and 11 for **10**) in common with the most potent and selective PPPA derivative **2**-**4**, the SOAT2 inhibitory activity may be maintained. Therefore, their substituents proved to be considerably effective for binding to SOAT2. Unfortunately, the isozyme selectivities of analogs **7** and **10,** exhibiting a potent SOAT2 inhibitory activity, were less than that of **1**. With regard to isozyme selectivity, the rigid steroid-like skeleton of **1** might be better than the A-ring simplified PPPA analogs. However, notably, the analogs **7** and **10** have the highest SI values among all synthetic SOAT inhibitors reported till date.



Scheme 3. Synthesis of A-ring simplified PPPA analog **8**. *Reagents and conditions*: a) ref. 40; b) 1. *t*-BuOCl, silica gel, hexane, –30 °C to rt, 92%, 2. Cu_2O, *p*-TsOH, DMSO, H_2O, rt, 92%; c) 1. *m*CPBA, CH_2Cl_2, 0 °C to rt, 99%, 2. TBSCl, imidazole, CH_2Cl_2, rt, 95%; d) 1. Cp_2TiCl_2, Mn, H_2O, THF, rt, 72%, 2. TBSCl, imidazole, CH_2Cl_2, rt, 93%; e) 1. NBA, AgOAc, AcOH, rt, 2. K_2CO_3, MeOH, rt, 60% over two steps; f) 1. $TMSCH_2OMe$, *s*-BuLi, *t*-BuOK, THF, –78 °C to 0 °C, 2. TBSCl, Et_3N, DMAP, DMF, 50 °C; g) 1. **15**, *i*-PrMgCl, THF, –30 °C to rt, 2. DMP, CH_2Cl_2, 0 °C, 42% over four steps; h) 1. MeOH, toluene, 90 °C, 2. DBU, toluene, 100 °C, 66% over two steps; i) LHMDS, nicotinoyl chloride hydrochloride, THF, –78 °C to rt, 34%; j) 1. AcCl, MeOH, 0 °C, 2. Ac_2O, Et_3N, DMAP, MeCN, 0 °C to rt, 3. $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 51% over three steps.



Scheme 4. Synthesis of A-ring simplified PPPA analogs **9** and **10**. *Reagents and conditions*: a) 1. AcCl, MeOH, 0 °C to rt, 2. $(t\text{-Bu})_2Si(OTf)_2$, 2,6-lutidine, DMF, 0 °C, 3. *p*-cyanobenzoic acid, EDCI, DMAP, CH_2Cl_2, rt; b) 1. $Et_3N{\cdot}3HF$, THF, rt, 2. Ac_2O, Et_3N, DMAP, MeCN, 0 °C to rt, 3. $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 38% over six steps; c) 1. AcCl, MeOH, 0 °C, 2. *o*-tolualdehyde, PPTS, DMF, 0 °C to rt; d) 1. *p*-cyanobenzoic acid, EDCI, DMAP, DMF, rt, 2. $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 25% over four steps.



(*S*)-Carvone **40**

Figure 4. Structure of the A-ring simplified PPPA analog **40** reported by Li and Nan et al. as a potent and selective SOAT2 inhibitor.[17]

Table 2. SOAT1 and SOAT2 inhibitory activities and isozyme selectivities of A-ring simplified PPPA analogs **5**–**10**, **21**, **22**, and **40**.

	IC_{50} (μM)		
Compounds	SOAT1	SOAT2	SI[a]
Pyripyropene A (**1**)	>80.0	0.07	>1000.0
5	>23.5	>23.50	—
21	>23.5	>23.50	—
22	>23.5	>23.50	—
6	>22.0	3.40	6.5
7	8.0	0.07	114.3
8	>17.4	>17.40	—
9	>15.1	0.86	17.6
10	9.8	0.11	89.1
40	>18.4	>18.40	—

[a] Selectivity index (SI): IC_{50}(SOAT1) / IC_{50}(SOAT2)

Conversely, A-ring simplified PPPA analog **40** reported by Li and Nan et al. as a potent and selective SOAT2 inhibitor did not exhibit any SOAT2 inhibitory activity and isozyme selectivity in our assay system, which not only is different from that used in their study but also is the most accurate assay system used currently for measuring the SOAT2 inhibitory activity and isozyme selectivity.[21] The new analogs **7** and **10** were considered to be not only the most potent and selective synthetic SOAT2 inhibitor but also attractive seed compounds for the discovery of drugs for dyslipidemia, including atherosclerosis and steatosis.

Conclusions

In conclusion, novel A-ring simplified PPPA analogs were designed, synthesized, and evaluated in terms of their SOAT2 inhibitory activities and isozyme selectivities. Designed analogs were prepared by the application of the total synthesis of PPPA reported by our group[15] except for several steps associated with the A-ring construction. Among the analogs, the synthesized A-ring simplified pyripyropene A analogs **7** and **10** exhibited the same efficacy for the SOAT2 inhibitory activity compared with that of natural pyripyropene A and extremely high isozyme selectivity as synthetic compounds. Currently, the further optimization of A-ring simplified PPPA analogs and *in vivo* tests are underway in our laboratory.

Experimental Section

Chemistry

General: All reactions were performed in flame-dried glassware under nitrogen using standard techniques for handling air-sensitive materials. Commercial reagents were used without further purification unless otherwise noted. Organic solvents were distilled and dried over 3 or 4 Å molecular sieves (MS). Cold baths were prepared under the following conditions: 0 °C, wet ice/water; −78 °C, dry ice/acetone. Purification by flash column chromatography was performed over silica gel 60N (spherical, neutral, particle size of 40–50 μm). Thin-layer chromatography (TLC) was performed on 0.25 mm Merck silica gel 60 F254 plates, and the effluents were visualized by UV (254 nm) as well as using phosphomolybdic acid and *p*-anisaldehyde TLC stains. Yields corresponded to chromatographically and spectroscopically pure compounds unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded using an internal deuterium lock on 400-MR, VNMRS-400, and UNITY-400 spectrometers (Agilent Technologies, Waldbronn, Germany). All NMR signals were reported in ppm relative to the internal reference standard provided by chloroform (i.e., 7.26 or 77.0 ppm for ^{1}H and ^{13}C spectra, respectively). Multiplicity data were presented as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, and dt = doublet of triplets. Coupling constants (*J*) were reported in hertz. Infrared spectra were recorded on an FT/IR460-plus infrared spectrometer (JASCO, Tokyo, Japan). Absorption data were expressed in wavenumbers (cm^{-1}). Optical rotation was recorded on a JASCO DIP-1000 polarimeter (JASCO, Tokyo, Japan) and reported as follows: $[\alpha]^T_D$, concentration (g/100 mL), and solvent. High-resolution mass spectra were recorded on a JEOL JMS-700 MStation, JEOL JMS-AX505HA, and JEOL JMS-T100LP systems (JEOL, Tokyo, Japan) equipped with FAB, EI, and ESI high-resolution mass spectrometers.

(1*S*,4*R*,6*S*)-4-Isopropyl-1-methyl-7-oxabicyclo[4.1.0]heptan-2-one (13): Colorless solid; $[\alpha]^{27}_D$ –9.2 (*c* 1.0, $CHCl_3$); IR (KBr) 1711, 1215 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.43 (d, 1H, *J* = 4.8 Hz), 2.56 (t, 1H, *J* = 13.2 Hz), 2.11-2.03 (m, 2H), 1.89-1.81 (m, 2H), 1.55-1.48 (m, 1H), 1.39 (s, 3H), 0.90 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 209.7, 65.7, 59.9, 44.8, 39.4, 32.8, 27.3, 19.5, 19.3, 15.1; EI-HRMS calcd for $C_{10}H_{16}O_2$ 168.1150 (M^+), found 168.1148 (M^+).

(3*S*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-isopropyl-2-methylcyclohex-1-enecarbaldehyde (14): Colorless solid; $[\alpha]^{27}_D$ +53.5 (*c* 1.0, $CHCl_3$); IR (KBr) 3054, 2959, 1710, 1680, 1423, 1265 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 4.27 (br s, 1H), 2.41-2.35 (m, 1H), 2.14 (s, 3H), 2.04-1.96 (m, 1H), 1.78-1.68 (m, 1H), 1.61-1.49 (m, 1H), 1.33-1.24 (m, 1H), 1.26 (dd, 1H, *J* = 10.0, 11.2 Hz), 0.92 (s, 9H) 0.90 (dd, 6H, *J* = 1.8, 6.6 Hz), 0.12 (d, 6H, *J* = 4.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 192.5, 157.2, 134.3, 73.7, 38.2, 36.7, 32.5, 26.4, 26.1, 20.0, 19.5, 18.4, 13.7, –3.6, –4.6; FAB-HRMS (*m*-NBA) calcd for $C_{17}H_{32}NaO_2Si$ 319.2069 ($M+Na^+$), found 319.2064 ($M+Na^+$).

5-((3*S*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-isopropyl-2-methylcyclohex-1-enecarbonyl)-2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (16): Colorless oil; $[\alpha]^{27}_D$ +26.6 (*c* 1.0, $CHCl_3$); IR (KBr) 3120, 2958, 1734, 1658, 1571, 1463, 1390, 1352, 1260, 1216, 1076 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.21 (br s, 1H), 2.27 (s, 3H), 2.15-2.08 (m, 1H), 1.96-1.90 (m, 2H), 1.70 (br s, 9H), 1.53-1.46 (m, 2H), 1.34-1.28 (m, 1H), 0.87 (br s, 15H), 0.06 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 196.8, 175.4, 158.8, 138.5, 136.1, 109.8, 106.6, 73.0, 39.1, 36.7, 32.4, 31.3, 31.1, 26.2, 25.8, 25.7, 20.2, 19.9, 19.7, 18.5, 16.5, –3.7, –4.5; ESI-HRMS (TFA-Na) calcd for $C_{24}H_{40}NaO_5Si$, 459.2543 ($M+Na^+$), found 459.2526 ($M+Na^+$).

(4a*R*,6*S*,8*S*,8a*S*)-Methyl-8-((*tert*-butyldimethylsilyl)oxy)-6-isopropyl-2,8a-dimethyl-4-oxo-4a,5,6,7,8,8a-hexahydro-4*H*-chromene-3-carboxylate (17): As a 3 : 1 diastereomixture; colorless oil; $[\alpha]^{27}_{D}$ +31.7 (*c* 1.0, $CHCl_3$); IR (KBr) 2957, 2932, 1707, 1679, 1587, 1464, 1437, 1391, 1362, 1257, 1216, 1114 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.87-3.82 (m, 1H), 3.79 (s, 3H), 2.47 (dd, 1H, *J* = 16.0, 4.0 Hz), 2.22 (s, 3H), 2.15-2.07 (m, 1H), 1.77-1.71 (m, 1H), 1.57-1.47 (m, 2H), 1.30-1.25 (m, 1H), 1.19 (s, 3H), 1.00-0.84 (m, 16H), 0.11 (d, 6H, *J* = 9.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.4, 175.3, 166.0, 110.3, 87.5, 76.3, 69.0, 52.2, 50.1, 40.6, 36.1, 32.4, 25.4, 23.6, 20.6, 19.7, 19.4, 17.9, 9.8, –4.8, –5.3; ESI-HRMS (TFA-Na) calcd for $C_{22}H_{38}NaO_5Si$, 433.2386 ($M+Na^+$), found 433.2403 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*R*)-6-((*tert*-Butyldimethylsilyl)oxy)-8-isopropyl-5a-methyl-3-(pyridin-3-yl)-5a,6,7,8,9,9a-hexahydropyrano[4,3-b]chromene-1,10-dione (18): As a 3 : 1 diastereomixture; colorless oil; $[\alpha]^{27}_{D}$ +11.1 (*c* 1.0, $CHCl_3$); IR (KBr) 3055, 2982, 2307, 1758, 1429, 1265 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.06 (d, 1H, *J* = 2.4 Hz), 8.75 (dd, 1H, *J* = 1.6, 4.8 Hz), 8.19 (dt, 1H, *J* = 2.0, 8.0 Hz), 7.46 (ddd, 1H, *J* = 2.0, 4.8, 8.0 Hz), 6.43 (s, 1H), 3.97 (dd, 1H, *J* = 5.3, 11.2 Hz), 2.63 (dd, 1H, *J* = 4.0, 12.0 Hz), 2.20 (dd, 1H, *J* = 2.0, 14.0 Hz), 1.81 (dt, 1H, *J* = 2.4, 13.2 Hz), 1.56 (dd, 1H, *J* = 6.8, 12.4 Hz), 1.48-1.40 (m, 1H), 1.30 (s, 3H), 1.22-1.21 (m, 1H), 1.11-1.05 (m, 1H), 0.96 (s, 9H), 0.92 (d, 3H, *J* = 6.8 Hz), 0.91 (d, 3H, *J* = 6.8 Hz), 0.19 (s, 3H), 0.15 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.0, 173.5, 162.7, 157.0, 152.8, 147.8, 134.1, 127.0, 134.1, 127.0, 124.1, 98.3, 89.9, 76.1, 51.6, 40.4, 36.0, 32.3, 30.0, 26.1, 24.3, 20.1, 19.0, 11.1, –4.1, –4.2; ESI-HRMS (TFA-Na) calcd for $C_{27}H_{37}NNaO_5Si$ 506.2339 ($M+Na^+$), found 506.2345 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*R*)-6-(Acetoxy)-8-isopropyl-5a-methyl-3-(phenyl)-5a,6,7,8,9,9a-hexahydropyrano[4,3-b]chromene-1,10-dione (19): Yellow solid; $[\alpha]^{27}_{D}$ +10.2 (*c* 1.0, $CHCl_3$); IR (KBr) 2930, 1757, 1628, 1536. 1431, 1262 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.06 (dd, 1H, *J* = 0.6, 2.1 Hz), 8.74 (dd, 1H, *J* = 1.8, 4.8 Hz), 8.18 (ddd, 1H, *J* = 1.8, 2.1, 8.4 Hz), 7.44 (dd, 1H, *J* = 0.6, 8.4 Hz), 6.54 (s, 1H), 5.26 (dd, 1H, *J* = 5.1, 11.7 Hz), 2.75 (dd, 1H, *J* = 3.6, 12.3 Hz), 2.27 (ddd, 1H, *J* = 3.9, 5.7, 14.4 Hz), 2.19 (s, 3H), 2.06-1.98 (m, 1H), 1.64-1.53 (m, 1H), 1.39 (s, 3H), 1.40-1.29 (m, 1H), 1.18-1.05 (m, 1H), 0.87-0.81 (m, 1H), 0.92 (d, 6H, *J* = 6.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.0, 173.2, 170.5, 162.9, 156.9, 153.0, 147.9, 134.1, 126.9, 124.1, 100.5, 98.5, 87.4, 76.1, 51.6, 40.4, 32.3, 32.0, 24.5, 21.6, 20.2, 20.0, 12.0; ESI-HRMS (TFA-Na) calcd for $C_{23}H_{25}NNaO_6$ 434.1580 ($M+Na^+$), found 434.1565 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*S*)-6-(Acetoxy)-8-isopropyl-5a-methyl-3-(phenyl)-5a,6,7,8,9,9a-hexahydropyrano[4,3-b]chromene-1,10-dione (20): Yellow solid; $[\alpha]^{27}_{D}$ +63.5 (*c* 1.0, $CHCl_3$); IR (KBr) 2930, 1757, 1628, 1536, 1431, 1262 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.08-9.06 (m, 1H), 8.75-8.72 (m, 1H), 8.22 (dd, 1H, *J* = 1.5, 7.2 Hz), 7.65 (dd, 1H, *J* = 5.1, 6.9 Hz), 6.41 (s, 1H), 5.38 (dd, 1H, *J* = 5.1, 11.7 Hz), 2.94 (dd, 1H, *J* = 3.0, 4.2 Hz), 2.67 (dd, 1H, *J* = 2.1, 13.2 Hz), 2.09 (s, 3H), 1.91-1.85 (m, 1H), 1.67 (s, 3H), 1.54-1.35 (m, 2H), 1.35-1.09 (m, 2H), 0.90 (d, 6H, *J* = 5.1 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 186.7, 173.6, 170.3, 162.8, 157.0, 152.4, 147.5, 134.6, 129.1, 124.3, 100.2, 98.7, 88.0, 70.0, 51.6, 37.7, 33.0, 32.4, 30.0, 25.7, 21.6, 21.4, 20.7; ESI-HRMS (TFA-Na) calcd for $C_{23}H_{25}NNaO_6$ 434.1588 ($M+Na^+$), found 434.1565 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*S*,10*R*)-10-Hydroxy-8-isopropyl-5a-methyl-1-oxo-3-(pyridin-3-yl)-1,5a,6,7,8,9,9a,10-octahydropyrano[4,3-b]chromen-6-yl acetate (5): Colorless solid; $[\alpha]^{27}_{D}$ +17.8 (*c* 1.0, $CHCl_3$); IR (KBr) 3055, 2929, 1708, 1428, 1264 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.03 (d, 1H, *J* = 4.8 Hz), 8.70 (d, 1H, *J* = 4.5 Hz), 8.15 (d, 1H, *J* = 5.1 Hz), 7.44 (dd, 1H, *J* = 3.0, 4.8 Hz), 6.50 (s, 1H), 5.02 (dd, 1H, *J* = 4.8, 11.7 Hz), 4.64 (d, 1H, *J* = 4.2 Hz), 2.21 (s, 3H), 2.01-1.95 (m, 1H), 1.90-1.81 (m, 2H), 1.65-1.42 (m, 3H), 1.50 (s, 3H), 1.37-1.29 (m, 1H), 0.94 (d, 3H, *J* = 6.6 Hz), 0.93 (d, 3H, *J* = 6.6 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.8, 164.4, 163.4, 157.7, 151.9, 147.2, 133.4, 103.6, 99.9, 83.0, 62.0, 43.7, 41.4, 32.6, 30.1, 27.8, 23.0, 21.7, 20.2, 20.1, 14.5, 13.0; ESI-HRMS (TFA-Na) calcd for $C_{23}H_{27}NNaO_6$ 436.1736 ($M+Na^+$), found 436.1723 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*S*,10*S*)-10-Hydroxy-8-isopropyl-5a-methyl-1-oxo-3-(pyridin-3-yl)-1,5a,6,7,8,9,9a,10-octahydropyrano[4,3-b]chromen-6-yl acetate (21): Colorless solid; $[\alpha]^{27}_{D}$ +27.1 (*c* 1.0, $CHCl_3$); IR (KBr) 2928, 2859, 1715, 1261 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.01 (d, 1H, *J* = 2.4 Hz), 8.69 (dd, 1H, *J* = 1.6, 4.8 Hz), 8.10 (ddd, 1H, *J* = 1.6, 2.4, 8.0 Hz), 7.40 (ddd, 1H, *J* = 0.8, 5.2, 8.0 Hz), 6.49 (s, 1H), 5.06 (dd, 1H, *J* = 4.8, 12.0 Hz), 4.45 (d, 1H, *J* = 10.0 Hz), 2.33-2.27 (m, 1H), 2.18 (s, 3H), 2.01-1.95 (m, 1H), 1.86 (ddd, 1H, *J* = 3.6, 10.0, 12.4 Hz), 1.59-1.43 (m, 2H), 1.33-1.27 (m, 1H), 1.28 (s, 3H), 1.37-1.20 (m, 1H), 1.02-0.87 (m, 1H), 0.93 (d, 3H, *J* = 6.7 Hz), 0.91 (d, 3H, *J* = 6.7 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 164.0, 163.4, 157.6, 151.2, 146.8, 133.7, 121.0, 100.0, 83.9, 64.5, 63.6, 45.0, 40.9, 32.4, 30.0, 28.9, 23.0, 21.7, 20.2, 20.1, 14.5, 12.3; ESI-HRMS (TFA-Na) calcd for $C_{23}H_{27}NNaO_6$ 436.1736 ($M+Na^+$), found 436.1734 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*R*,10*R*)-10-Hydroxy-8-isopropyl-5a-methyl-1-oxo-3-(pyridin-3-yl)-1,5a,6,7,8,9,9a,10-octahydropyrano[4,3-b]chromen-6-yl acetate (22): Colorless solid; $[\alpha]^{27}_{D}$ +18.5 (*c* 0.1, $CHCl_3$); IR (KBr) 2927, 1715, 1572, 1432, 1223, 1094 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.02-9.00 (m, 1H), 8.70-8.68 (m, 1H), 8.16 (d, 1H, *J* = 8.0 Hz), 7.46 (dd, 1H, *J* = 4.8, 7.2 Hz), 6.42 (s, 1H), 5.04 (dd, 1H, *J* = 5.2, 12.0 Hz), 4.77 (d, 1H, *J* = 9.6 Hz), 2.21-2.14 (m, 2H), 2.09 (s, 3H), 1.91-1.85 (m, 1H), 1.59-1.35 (m, 3H), 1.54 (s, 3H), 1.33-1.22 (m, 1H), 0.94 (d, 3H, *J* = 6.8 Hz), 0.92 (d, 3H, *J* = 6.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5, 164.1, 162.9, 156.9, 150.7, 146.3, 134.4, 102.6, 100.2, 84.4, 70.8, 68.5, 60.1, 44.6, 37.0, 32.5, 30.0, 27.4, 21.6, 21.0, 20.5, 20.2, 20.1; ESI-HRMS (TFA-Na) calcd for $C_{23}H_{28}NO_6$ 414.1917 (MH^+), found 414.1910 (MH^+).

(1*S*,4*S*,6*S*)-4-Isopropyl-1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one (24): Yellow oil; $[\alpha]^{27}_{D}$ –102.7 (*c* 1.0, $CHCl_3$); IR (KBr) 1743, 1075 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.42 (d, 1H, *J* = 4.8 Hz), 2.15 (dd, 1H, *J* = 10.4, 15.6 Hz), 2.02 (ddd, 1H, *J* = 4.8, 6.4, 15.6 Hz), 1.96-1.89 (m, 1H), 1.58-1.53 (m, 1H), 1.38 (s, 3H), 1.16 (s, 3H), 1.12 (s, 3H), 0.93 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 210.5, 63.3, 57.1, 49.9, 47.5, 27.6, 24.8, 24.4, 20.9, 20.8, 18.9, 16.7; EI-HRMS calcd for $C_{12}H_{20}O_2$ 196.1463 (M^+), found 196.1451 (M^+).

5-((3*S*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-isopropyl-2,6,6-trimethylcyclohex-1-ene-1-carbonyl)-2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (26): Yellow oil; $[\alpha]^{27}_{D}$ +27.7 (*c* 1.0, $CHCl_3$); IR (KBr) 2957, 2858, 1744, 1656, 1545, 1468, 1377, 1346, 1253, 1198 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.15 (t, 1H, *J* = 14.7 Hz), 2.45 (s, 3H), 1.98-1.89 (m, 1H), 1.79-1.71 (m, 1H), 1.70 (s, 3H), 1.69 (s, 3H), 1.60-1.55 (m, 2H), 1.55 (s, 3H), 1.13 (s, 3H), 0.95 (s, 3H), 0.93 (d, 3H, *J* = 6.9 Hz), 0.89 (s, 9H), 0.84 (d, 3H, *J* = 6.9 Hz), 0.10 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 197.6, 179.1, 158.2, 143.4, 132.4, 110.3, 105.8, 77.4, 72.6, 47.3, 39.5, 29.7, 26.0, 25.6, 25.3, 25.3, 25.2, 24.8, 24.6, 23.7, 21.8, 18.8, 18.3, 16.5, –3.8, –4.7; ESI-HRMS (TFA-Na) calcd for $C_{26}H_{44}NaO_5Si$ 487.2856 ($M+Na^+$), found 487.2841 ($M+Na^+$).

(4aR,6S,8S,8aS) -Methyl-8-((*tert*-butyldimethylsilyl)oxy)-6-isopropyl-2,5,5,8a-tetramethyl-4-oxo-4a,5,6,7,8,8a-hexahydro-4*H*-chromene-3-carboxylate (27): Yellow oil; $[\alpha]^{27}_{D}$ +19.9 (*c* 0.1, $CHCl_3$); IR (KBr) 2371, 1745, 1060 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.82 (dd, 1H, *J* = 4.8, 11.6 Hz), 3.78 (s, 3H), 2.39 (s, 1H), 2.13 (s, 3H), 2.02-1.97 (m, 1H), 1.52 (ddd, 1H, *J* = 3.2, 4.8, 13.7 Hz), 1.39-1.29 (m, 1H), 1.31 (s, 3H), 1.25 (s, 3H), 1.04-1.00 (m, 1H), 1.03 (s, 3H), 0.91 (s, 9H), 0.90 (d, 3H, *J* = 6.8 Hz), 0.79 (d, 3H, *J* = 6.8 Hz), 0.10 (s, 3H), 0.10 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.3, 174.0, 166.6, 111.5, 87.9, 77.2, 58.9, 52.0, 51.3, 37.8, 29.4, 28.9, 25.9, 25.2, 24.6, 20.6, 19.0, 18.3, 16.9, 13.5, –4.4; ESI-HRMS (TFA-Na) calcd for $C_{24}H_{42}NaO_5Si$ 461.2699 ($M+Na^+$), found 461.2697 ($M+Na^+$).

(5aS,6S,8S,9aR)-6-((*tert*-Butyldimethylsilyl)oxy)-8-isopropyl-5a,9,9-trimethyl-3-(pyridin-3-yl)-5a,6,7,8,9,9a-hexahydro-1H,10H-pyrano[4,3-b]chromene-1,10-dione (28): Yellow oil; $[\alpha]^{27}_{D}$ +43.1 (*c* 1.0, $CHCl_3$); IR (KBr) 2965, 1743, 1262, 1075 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.74 (d, 1H, *J* = 6.0 Hz), 8.17 (d, 1H, *J* = 13.2 Hz),

7.46-7.42 (m, 1H), 6.38 (s, 1H), 3.94 (dd, 1H, *J* = 4.8, 11.7 Hz), 2.55 (s, 1H), 2.06-1.99 (m, 1H), 1.62-1.56 (m, 2H), 1.30 (s, 3H), 1.24 (s, 3H), 1.11 (s, 3H), 1.11-1.06 (m, 1H), 0.95 (s, 9H), 0.93 (d, 3H, *J* = 6.8 Hz), 0.82 (d, 3H, *J* = 6.8 Hz), 0.18 (s, 3H), 0.15 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.5, 172.4, 161.9, 157.1, 151.9, 146.9, 134.5, 100.9, 98.1, 90.8, 90.5, 77.4, 76.9, 60.4, 51.1, 38.2, 29.8, 29.4, 28.8, 25.9, 25.2, 24.6, 22.8, 18.9, 18.3, 16.8, 14.2, –4.2, –4.3; ESI-HRMS (TFA-Na) $[M+Na]^+$ calcd for $C_{29}H_{41}NNaO_5$ 534.2652 ($M+Na^+$), found 534.2658 ($M+Na^+$).

(5aS,6S,8S,9aS,10R)-10-Hydroxy-8-isopropyl-5a,9,9-trimethyl-1-oxo-3-(pyridin-3-yl)-5a,6,8,9,9a,10-hexahydro-1H,7H-pyrano[4,3-b]chromen-6-yl acetate (6): Colorless solid; $[\alpha]^{27}_D$ +58.3 (*c* 1.0, $CHCl_3$); IR (KBr) 3433, 2361, 1638, 1241, 1041 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.70 (s, 1H), 8.13 (d, 1H, *J* = 8.4 Hz), 7.44 (brs, 1H), 6.47 (s, 1H), 5.05 (d, 1H, *J* = 4.0 Hz), 5.00 (dd, 1H, *J* = 4.8, 12.0 Hz), 2.82 (brs, 1H), 2.18 (s, 3H), 2.06-1.99 (m, 1H), 1.79 (ddd, 1H, *J* = 13.2, 4.8, 3.2 Hz), 1.67 (s, 3H), 1.54 (m, 2H), 1.32 (m, 1H), 1.28 (s, 3H), 1.23 (s, 3H), 0.96 (d, 3H, *J* = 6.8 Hz), 0.85 (d, 3H, *J* = 6.8 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.5, 161.7, 126.5, 105.0, 102.2, 84.3, 78.0, 77.4, 60.6, 53.0, 51.5, 39.8, 29.9, 27.8, 26.3, 25.6, 25.4, 21.6, 19.1, 19.1, 15.9, 15.8; ESI-HRMS (TFA-Na) calcd for $C_{25}H_{31}NNaO_6$ 464.2049 ($M+Na^+$), found 464.2048 ($M+Na^+$).

(5aS,6S,8S,9aS,10R)-10-Hydroxy-8-isopropyl-5a,9,9-trimethyl-1-oxo-3-(pyridin-3-yl)-5a,6,8,9,9a,10-hexahydro-1H,7H-pyrano[4,3-b]chromen-6-yl 4-cyanobenzoate (7): Colorless solid; $[\alpha]^{27}_D$ +88.3 (*c* 0.1, $CHCl_3$); IR (KBr) 3433, 3020, 2341, 1637, 1216 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.99 (brs, 1H), 8.68 (brs, 1H), 8.23 (d, 2H, *J* = 8.4 Hz), 8.14 (brs, 1H), 7.80 (d, 2H, *J* = 8.4 Hz), 7.45 (brs, 1H), 6.41 (s, 1H), 5.28 (dd, 1H, *J* = 5.0, 12.2 Hz), 5.09 (d, 1H, *J* = 3.6 Hz), 2.85 (brs, 1H, 2.10-2.04 (m, 1H), 1.92 (ddd, 1H, *J* = 3.2, 4.8, 13.0 Hz), 1.82 (s, 3H), 1.70 (m, 1H), 1.63 (d, 1H, *J* = 4.0 Hz), 1.28 (s, 3H), 1.25 (m, 4H), 0.98 (d, 3H, *J* = 6.8 Hz), 0.86 (d, 3H, *J* = 6.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.3, 163.8, 162.0, 134.3, 132.5, 130.5, 118.1, 116.8, 103.9, 100.2, 83.8, 79.8, 77.4, 77.3, 60.8, 53.1, 51.6, 39.7, 31.8, 31.1, 29.9, 27.7, 26.3, 25.6, 25.4, 22.9, 22.8, 19.1, 19.0, 16.1, 14.4; ESI-HRMS (TFA-Na) calcd for $C_{31}H_{32}N_2NaO_6$ 551.2158 ($M+Na^+$), found 511.2171 ($M+Na^+$).

(*S*)-5-(3-Hydroxyprop-1-en-2-yl)-2,6,6-trimethylcyclohex-2-enone (30): Yellow oil; $[\alpha]^{27}_D$ +176.1 (*c* 1.0, $CHCl_3$); IR (KBr) 3609, 3020, 1701, 1666, 1522, 1423, 1216 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) d 6.62 (m, 1H), 5.27 (s, 1H), 5.00 (s, 1H), 4.05 (m, 2H), 2.53 (m, 1H), 2.44 (m, 2H), 1.79 (m, 3H), 1.11 (s, 3H), 1.04 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 204.4, 149.0, 142.6, 133.6, 112.0, 66.6, 47.1, 44.8, 29.7, 23.8, 19.8, 16.4; EI-HRMS calcd for $C_{12}H_{18}O_2$ 194.1307 (M^+), found 194.1306 (M^+).

(*R*)-5-((*R*)-2-(((*tert*-Butyldimethylsilyl)oxy)methyl)oxiran-2-yl)-2,6,6-trimethylcyclohex-2-enone (31): As a 1 : 1 diastereomixture; Yellow oil; $[\alpha]^{27}_D$ –48.0 (*c* 1.0, $CHCl_3$); IR (KBr) 1633, 1106 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.58 (t, 1H, *J* = 2.4 Hz), 3.60 (m, 2H), 2.94 (d, 1H, *J* = 5.4 Hz), 2.61 (m, 1H), 2.61 (d, 1H, *J* = 2.7 Hz), 2.44 (m, 1H), 1.93 (dd, 1H, *J* = 5.1, 6.0 Hz), 1.76 (s, 3H), 1.21 (s, 3H), 1.19 (s, 3H), 0.86 (m, 9H), 0.01 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 203.7, 203.2, 141.9, 141.7, 133.9, 133.5, 64.3, 63.1, 61.3, 61.0, 52.7, 50.1, 49.3, 47.9, 44.6, 44.2, 26.5, 26.4, 26.1, 26.0, 25.8, 21.6, 21.3, 18.5, 18.4, 16.6, 16.5, –5.3, –5.4; ESI-HRMS (TFA-Na) calcd for $C_{18}H_{32}NaO_3Si$ 347.2018 ($M+Na^+$), found 347.2021 ($M+Na^+$).

(*S*)-2,6,6-Trimethyl-5-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)cyclohex-2-enone (32): Colorless oil; $[\alpha]^{27}_D$ –31.9 (*c* 1.0, $CHCl_3$); IR (KBr) 3020, 2955, 2930, 2360, 2340, 1660, 1470, 1255, 1216, 1088 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.59 (dd, 1H, *J* = 0.9, 1.8 Hz), 3.55 (m, 4H), 2.35 (m, 2H), 2.06 (m, 1H), 1.85 (m, 1H), 1.72 (s, 3H), 1.16 (s, 3H), 1.02 (s, 3H), 0.86 (m, 18H), 0.01 (m, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 205.0, 143.7, 133.4, 63.8, 61.4, 45.6, 43.4, 42.9, 26.1, 24.8, 23.9, 20.5, 18.4, 16.6, –5.3, –5.4; ESI-HRMS (TFA-Na) calcd for $C_{24}H_{48}NaO_3Si_2$ 463.3040 ($M+Na^+$), found 463.3061 ($M+Na^+$).

(1*S*,4*S*,6*S*)-1,3,3-Trimethyl-4-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)-7-oxabicyclo[4.1.0]heptan-2-one (33): Colorless solid; $[\alpha]^{27}_D$ –33.8 (*c* 1.0, $CHCl_3$); IR (KBr) 1701, 1470, 1406, 1389, 1362, 1255, 1216, 1091, 1007 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.60 (m, 4H), 3.38 (ddd, 1H, *J* = 1.2, 1.5, 3.0 Hz), 2.17 (m, 2H), 1.89 (m, 1H), 1.74 (m, 1H), 1.38 (s, 3H) 1.17 (s, 3H), 1.11 (s, 3H), 0.88 (m, 18H), 0.04 (m, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 210.4, 63.7, 63.4, 61.2, 57.2, 47.3, 43.3, 43.2, 26.1, 24.8, 22.5, 18.4, 16.8, –5.2, –5.3; ESI-HRMS (TFA-Na) calcd for $C_{24}H_{48}NaO_4Si_2$ 479.2989 ($M+Na^+$), found 479.2967 ($M+Na^+$).

5-((3*S*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2,6,6-trimethyl-5-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)cyclohex-1-enecarbonyl)-2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (35): Yellow oil; $[\alpha]^{27}_D$ –11.0 (*c* 1.0, $CHCl_3$); IR (KBr) 3020, 2956, 2930, 1739, 1649, 1538, 1471, 1380, 1349, 1255, 1216 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.12 (ddd, 1H, *J* = 2.7, 6.0, 6.6 Hz), 3.61 (m, 4H), 2.44 (s, 3H), 1.92 (m, 2H), 1.75 (m, 1H), 1.70 (s, 3H), 1.69 (s, 3H), 1.62 (m, 1H), 1.52 (d, 3H, *J* = 9.2 Hz) 1.15 (s, 3H), 0.97 (s, 3H), 0.89 (m, 27H), 0.04 (m, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 197.7, 178.9, 158.0, 143.3, 132.4, 110.3, 105.8, 72.2, 64.6, 61.0, 41.8, 41.5, 39.0, 30.7, 26.2, 26.1, 26.0, 25.5, 25.4, 25.1, 24.3, 23.6, 21.8, 18.5, 18.4, 18.2, 16.5, –3.8, –4.8, –5.3; ESI-HRMS (TFA-Na) $[M+Na]^+$ calcd for $C_{38}H_{72}NaO_7Si_3$ 747.4484 ($M+Na^+$), found 747.4481 ($M+Na^+$).

(4a*R*,6*S*,8*S*,8a*S*)-Methyl-8-((*tert*-butyldimethylsilyl)oxy)-2,5,5,8a-tetramethyl-6-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)-4-oxo-4a,5,6,7,8,8a-hexahydro-4*H*-chromene-3-carboxylate (36): Yellow oil; $[\alpha]^{27}_D$ –3.3 (*c* 1.0, $CHCl_3$); IR (KBr) 3021, 2955, 2931, 1636, 1390, 1256, 1215, 1103 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.79 (ddd, 4H, *J* = 4.5, 4.8, 6.6 Hz), 3.60 (m, 3H), 3.45 (dd, 1H, *J* = 8.4, 9.9 Hz), 2.43 (s, 1H), 2.14 (s, 3H), 1.82 (d, 1H, *J* = 3.0 Hz), 1.69 (m, 1H), 1.50 (d, 1H, *J* = 13.5 Hz), 1.37 (d, 1H, *J* = 11.4 Hz), 1.32 (s, 3H), 1.06 (s, 3H), 0.92 (s, 3H), 0.88 (m, 27H), 0.04 (m, 18H).; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.3, 174.0, 166.6, 111.5, 87.8, 64.5, 61.2, 58.7, 52.1, 45.1, 40.7, 37.3, 30.4, 28.7, 26.2, 26.1, 25.9, 20.6, 18.5, 18.3, 16.8, 13.7, –4.4, –5.2, –5.3; ESI-HRMS (TFA-Na) calcd for $C_{36}H_{70}NaO_7Si_3$ 721.4327 ($M+Na^+$), found 721.4349 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*R*)-6-((*tert*-Butyldimethylsilyl)oxy)-5a,9,9-trimethyl-8-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)-3-(pyridin-2-yl)-5a,6,7,8,9,9a-hexahydropyrano[4,3-*b*]chromene-1,10-dione (37): Yellow oil; $[\alpha]^{27}_D$ +21.9 (*c* 1.0, $CHCl_3$); IR (KBr) 3025, 2955, 2931, 2858, 1756, 1630, 1543, 1479, 1442, 1422, 1257, 1094 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.76 (s, 1H), 8.22 (d, 1H, *J* = 8.1 Hz), 7.50 (m, 1H), 6.39 (s, 1H), 3.79 (ddd, 4H, *J* = 5.1, 5.7, 8.7 Hz), 3.50 (m, 1H), 2.60 (s, 1H), 1.84 (m, 1H), 1.71 (m, 1H), 1.56 (m, 1H), 1.42 (m, 1H), 1.44 (s, 3H), 1.15 (s, 3H), 0.97 (s, 3H), 0.91 (m, 27H), 0.04 (m, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.4, 172.4, 162.3, 157.2, 152.5, 147.5, 134.1, 126.9, 124.0, 100.9, 97.8, 90.4, 64.5, 61.2, 60.3, 45.0, 40.7, 37.8, 30.5, 28.6, 26.2, 26.1, 26.0, 18.6, 18.4, 16.7, 14.4, –4.2, –5.2, –5.3; ESI-HRMS (TFA-Na) calcd for $C_{41}H_{70}NO_7Si_3$ 772.4460 (MH^+), found 772.4446 (MH^+).

2-((5a*S*,6*S*,8*S*,9a*S*,10*R*)-6-Acetoxy-10-hydroxy-5a,9,9-trimethyl-1-oxo-3-(pyridin-2-yl)-1,5a,6,7,8,9,9a,10-octahydropyrano[4,3-*b*]chromen-8-yl)propane-1,3-diyl diacetate (8): Colorless solid; $[\alpha]^{27}_D$ –145.2 (*c* 0.1, $CHCl_3$); IR (KBr) 3445, 2930, 2360, 2340, 1735, 1697, 1643, 1584, 1373, 1250, 1041 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.71 (s, 1H), 8.20 (m, 1H), 7.50 (m, 1H), 6.50 (d, 1H, *J* = 4.8 Hz), 5.05 (d, 1H, *J* = 4.0 Hz), 4.94 (m, 1H), 4.19 (m, 2H), 3.97 (m, 2H), 2.29 (m, 1H), 2.16 (m, 3H), 2.12 (m, 2H), 2.08 (m, 1H), 2.08 (m, 6H), 1.97 (m, 1H), 1.58 (s, 3H), 1.34 (s, 3H), 1.25 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 211.2, 157.7, 155.4, 154.3, 154.0, 153.4, 152.2, 136.0, 121.1, 113.7, 111.3, 69.6, 68.3, 60.0, 56.6, 42.2, 37.6, 33.6, 33.5, 33.4, 33.2, 21.8, 18.3, 18.1, 18.0, 16.3, 12.2, 12.0; ESI-HRMS (TFA-Na) calcd for $C_{29}H_{35}NNaO_{10}$ 580.2159 ($M+Na^+$), found 580.2137 ($M+Na^+$).

2-((5aS,6S,8S,9aS,10R)-6-((4-Cyanobenzoyl)oxy)-10-hydroxy-5a,9,9-trimethyl-1-oxo-3-(pyridin-3-yl)-5a,6,8,9,9a,10-hexahydro-1H,7H-pyrano[4,3-b]chromen-8-yl)propane-1,3-diyl diacetate (9): Colorless solid; $[\alpha]^{27}_{D}$ +97.2 (*c* 0.1, $CHCl_3$); IR (neat) 3421, 3021, 2965, 1724, 1643, 1581, 1276, 1229, 1103, 1040 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.97 (brs, 1H), 8.67 (brs, 1H), 8.19 (d, 1H, *J* = 8.4 Hz), 8.06 (d, 1H, *J* = 8.0 Hz), 7.80 (d, 2H, *J* = 8.4 Hz), 7.38 (dd, 1H, *J* = 4.8, 8.0 Hz), 6.39 (s, 1H), 5.21 (dd, 1H, *J* = 4.4, 11.2 Hz), 5.08 (d, 1H, *J* = 4.0 Hz), 4.23-4.18 (m, 2H), 4.02 (dd, 1H, *J* = 6.4, 11.2 Hz), 3.93 (t, 1H, *J* = 10.0 Hz), 2.98 (brs, 1H), 2.35-2.31 (m, 1H), 2.10 (s, 3H), 2.06 (s, 3H), 1.82 (s, 3H), 1.77-1.65 (m, 3H), 1.37 (s, 3H), 1.27 (s, 3H), 1.27-1.23 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 164.0, 163.8, 161.8, 157.4, 151.5, 146.7, 133.9, 133.0, 132.4, 130.2, 123.6, 117.9, 116.8, 103.2, 99.1, 83.6, 82.8, 78.9, 65.6, 62.4 60.5, 52.5, 46.1, 39.1, 34.9, 27.1, 26.7, 24.8, 20.9, 18.5, 15.9, 15.0; ESI-HRMS (TFA-Na) calcd for $C_{35}H_{36}N_2NaO_{10}$ 667.2259 ($M+Na^+$), found 667.2268 ($M+Na^+$).

(5a*S*,6*S*,8*S*,9a*S*,10*R*)-10-Hydroxy-5a,9,9-trimethyl-1-oxo-3-(pyridin-3-yl)-8-(2-(o-tolyl)-1,3-dioxan-5-yl)-1,5a,6,7,8,9,9a,10-octahydropyrano[4,3-b]chromen-6-yl-4-cyanobenzoate (10): Colorless solid; $[\alpha]^{27}_{D}$ –105.6 (*c* 0.1, $CHCl_3$); IR (KBr) 3448, 2360, 2341, 1643, 1277, 1086 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) δ 9.08 (brs, 1H), 8.69 (s, 1H), 8.31 (d, 1H, *J* = 8.0 Hz), 8.23 (d, 2H, *J* = 8.0 Hz), 7.90 (d, 2H, *J* = 8.8 Hz), 7.58 (brs, 1H), 7.48 (dd, 1H, *J* = 1.6, 7.6 Hz), 7.19-7.08 (m, 3H), 6.77 (s, 1H), 5.55 (s, 1H), 5.22 (dd, 1H, *J* = 4.8, 12.0 Hz), 5.04 (d, 1H, *J* = 3.6 Hz), 4.27-4.23 (m, 1H), 4.00-3.96 (m, 1H), 3.81 (dd, 1H, *J* = 11.2, 18.8 Hz), 3.78 (dd, 1H, *J* = 11.2, 18.8 Hz), 2.42-2.32 (m, 1H), 2.31 (s, 3H), 2.12-2.06 (m, 1H), 1.86 (s, 3H), 1.80 (dd, 1H, *J* = 13.2, 25.6 Hz), 1.71 (d, 1H, *J* = 3.6 Hz), 1.40 (s, 3H), 1.32 (s, 3H), 1.27-1.22 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.3, 163.6, 161.7, 135.9, 135.7, 133.9, 132.6, 130.5, 130.4, 129.0, 126.1, 125.7, 117.9, 117.0, 103.8, 100.0, 82.9, 79.0, 72.0, 70.7, 60.5, 52.6, 45.7, 39.4, 32.8, 32.0, 29.8, 29.7, 29.5, 27.3, 27.2, 22.8, 19.0, 18.8, 16.0, 14.2; ESI-HRMS (TFA-Na) calcd for $C_{39}H_{39}N_2O_8$ 663.2706 (MH^+), found 663.2713 (MH^+).

Biological Assay Protocol

Cell Culture of SOAT1- and SOAT2-CHO Cells: Two cell lines, namely, CHO cells expressing SOAT1 and SOAT2 isozymes of African green monkey (SOAT1- and SOAT2-CHO cells, respectively),[11a] were gifted by Dr. L. L. Rudel (Wake Forest University, NC, USA). Briefly, both cell lines were maintained at 37 °C in 5% CO_2 in Ham's F-12 medium supplemented with MEM vitamins, Geneticin (300 μg/mL), and 10% heat-inactivated fetal bovine serum (hereafter referred to as medium A).

Assay for the Synthesis of Neutral Lipids in SOAT1- and SOAT2-CHO Cells: The assay for the synthesis of neutral lipids ([^{14}C]cholesteryl ester (CE), [^{14}C]triglyceride (TG), and [^{14}C]phospholipid (PL)) from [^{14}C]oleic acid in SOAT1- or SOAT2-CHO cells was carried out by our established method.[11b] Briefly, SOAT1- or SOAT2-CHO cells (1.25×10^5 cells in 250 μL of medium A) were cultured in a 48-well plastic microplate in the culture medium described above and allowed to recover overnight at 37 °C in 5% CO_2. The assays were carried out with cells that were at least 80% confluent. Following the overnight recovery, a test sample (2.5 μL; 0, 0.01, 0.1, and 1 mg/mL in MeOH) and [1-^{14}C]oleic acid (5 μL of a 10% EtOH/PBS solution, 1 nmol, 1.85 KBq) were added to each culture. After 6 h incubation at 37 °C in 5% CO_2, the medium was removed, and the cells in each well were washed twice with PBS. The cells were lysed by the addition of 0.25 mL of 10 mM Tris-HCl (pH 7.5) containing 0.1% (w/v) sodium dodecyl sulfate, and the cellular lipids were extracted by the method reported by Bligh and Dyer.[32] After concentrating the organic solvent, the total lipids were separated on a TLC plate (silica gel F254, 0.5 mm thickness, Merck, Darmstadt, Germany) and analyzed with an FLA7000 analyzer (Fuji Film). In this cell-based assay, [^{14}C]CE was produced by the reaction of SOAT1 or SOAT2. SOAT inhibitory activity (%) is defined as ([1-^{14}C]CE-drug/[^{14}C]CE-control) × 100. The IC_{50} value defined as the drug concentration causing 50% inhibition of the biological activity is calculated from duplicate experiments (*n* = 3).

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Conflict of interest

The authors declare no conflict of interest.

Keywords: A-ring simplified PPPA analog • pyripyropene A • SAR study • SOAT2 • Total synthesis

References:

[1] The older name for this enzyme is acyl-CoA: cholesterol acyltransferase or ACAT, but this acronym is also used for the enzyme acetyl-CoA acetyltransferase, so to avoid confusion we use SOAT to designate the cholesterol esterifying enzyme.

[2] B. D. Roth, *Drug Discov. Today*, **1998**, *3*, 19–25.

[3] J. C. Tardif, J. Gregoire, P. L. L'Allier, T. J. Anderson, O. Bertrand, F.Reeves, L. M. Title, F. Alfonso, E. Schampaert, A. Hassan, R. McLain, M. L. Pressler, R. Ibrahim, J. Lesperance, J. Blue, T. Heinonen, J. Rodes-Cabau, *Circulation,* **2004**, *110*, 3372–3377.

[4] S. E. Nissen, E. M. Tuzcu, H. B. Brewer, I. Sipahi, S. J. Nicholls, P. Ganz, P. Schoenhagen, D. D. Waters, C. J. Pepine, T. D. Crowe, M. H. Davidson, J. E. Deanfield, L. M. Winsniewski, J. J. Hanyok, L. M. Kassalow, *N. Engl. J. Med.*, **2006**, *354*, 1253–1263.

[5] T. Ohshiro, H. Tomoda, *Expert Opin. Ther.*, **2015**, *25*, 145–158.

[6] H. Tomoda, S. Omura, *Pharmacol. Ther.*, **2007**, *115*, 375–389.

[7] a) S. Omura, H. Tomoda, Y. K. Kim, H. Nishida, *J. Antibiot.*, **1993**, *46*, 1168–1169; b) H. Tomoda, Y. K. Kim, H. Nishida, R. Masuma, S. Omura, *J. Antibiot.*, **1994**, *47*, 148–153; c) Y. K. Kim, H. Tomoda, H. Nishida, T. Sunazuka, R. Obata, S. Omura, *J. Antibiot.*, **1994**, *47*, 154–162.

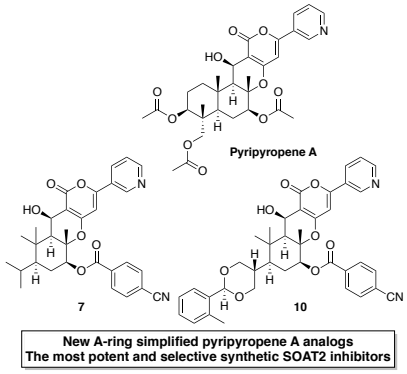
[8] a) R. Obata, T. Sunazuka, Z. Li, H. Tomoda, S. Omura, *J. Antibiot.*, **1995**, *48*, 749–750; b) R. Obata, T. Sunazuka, H. Tomoda, Y. Harigaya, S. Omura, *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2683–2688; c) R. Obata, T. Sunazuka, Z. Li, Z. Tian, Y. Harigaya, N. Tabata, H. Tomoda, S. Omura, *J. Antibiot.*, **1996**, *49*, 1133–1148; d) R. Obata, T. Sunazuka, Y. Kato, H. Tomoda, Y. Harigaya and S. Omura, *J. Antibiot.*, **1996**, *49*, 1149–1156; e) R. Obata, T. Sunazuka, Z. Tian, H. Tomoda, Y. Harigaya, S. Omura, *J. Antibiot.*, **1997**, *50*, 229–236; f) R. Obata, T. Sunazuka, Z. Tian, H. Tomoda, Y. Harigaya, S. Omura, A. B. Smith, III., *Chem. Lett.*, **1997**, *26*, 935–936.

[9] a) C. C. Chang, H. Y. Huh, K. M. Cadigan and T. Y. Chang, *J. Biol. Chem.*, **1993**, *268*, 20747–20755; b) R. A. Anderson, C. Joyce, M. Davis, J. W. Reagan, M. Clark, G. S. Shelness and L. L. Rudel, *J. Biol. Chem.*, **1998**, *273*, 26747–26754; c) S. Cases, S. Novak, Y. W. Zheng, H. M. Myers, S. R. Lear, E. Sande, C. B. Welch, A. J. Lusis, T. A. Spencer, B. R. Krause, S. K. Erickson and R. V. Farese, Jr. *J. Biol. Chem.*, **1998**, *273*, 26755–26764; d) P. Oelkers, A. Behari, D. Cromley, J. T. Billheimer and S. L. Sturley, *J. Biol. Chem.*, **1998**, *273*, 26765–26771.

[10] P. Parini, M. Davis, A. T. Lada, S. K. Erickson, T. L. Wright, U. Gustafsson, S. Sahlin, C. Einarsson, M. Eriksson, B. Angelin, H Tomoda, S. Omura, M. C. Willingham and L. L. Rudel, *Circulation*, **2004**, *110*, 2017–2023.

[11] a) A. T. Lada, M. Davis, C. Kent, J. Chapman, H. Tomoda, S. Omura and L. L. Rudel, *J. Lipid Res*., **2004**, *45*, 378–386; b) T. Ohshiro, L. L. Rudel, S. Omura and H. Tomoda, *J. Antibiot.*, **2007**, *60*, 43–51.
[12] a) T. Ohshiro, D. Matsuda, K. Sakai, C. Degirolamo, H. Yagyu, LL. Rudel, S. Omura, S. Ishibashi, H Tomoda, *Arterioscler. Thromb. Vasc. Biol*., **2011**, *31*, 1108–1115; b) T. Ohshiro and H. Tomoda, *Future Med. Chem.* **2011**, *3*, 2039–2061.
[13] a) M. Ohtawa, H. Yamazaki, S. Ohte, D. Matsuda, T. Ohshiro, L. L. Rudel, S. Omura, H. Tomoda and T. Nagamitsu, *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 1285–1287; b) M. Ohtawa, H. Yamazaki, D. Matsuda, T. Ohshiro, L. L. Rudel, S. Omura, H. Tomoda and T. Nagamitsu, *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 2659–2662; c) M. Ohtawa, H. Yamazaki, S. Ohte, D. Matsuda, T. Ohshiro, L. L. Rudel, S. Omura, H. Tomoda and T. Nagamitsu, *Bioorg. Med. Chem. Lett.,* **2013**, *23*, 3798–3801; d) M. Ohtawa, S. Omura, H. Tomoda and T. Nagamitsu, *J. Synth. Org. Chem. Jpn.,* **2013**, *71*, 830–843.
[14] T. Ohshiro, M. Ohtawa, T. Nagamitsu, D. Matsuda, H. Yagyu, H., M. A. Davis, L. L. Rudel, S. Ishibashi and H. Tomoda, *J. Pharm. Exp. Ther.,* **2015**, *355*, 299–307.
[15] A. Odani, K. Ishihara, M. Ohtawa, H. Tomoda, S. Omura and T. Nagamitsu, *Tetrahedron,* **2011**, *67*, 8195–8203.
[16] a) M. Ohtawa, S. Omura, T. Nagamitsu and H. Tomoda, Japan Patent Kokai 2014-144922 (2014.08.14); b) H. Tomoda, M. Ohtawa, S. Omura and T. Nagamitsu, WO 2015198966 2015.
[17] Y. Zhan, X-W. Zhang, Y. Xiong, B-L. Li and F-J. Nan, *Org. Biomol. Chem.*, **2016**, *14*, 747–751.
[18] W. D. Shipe and E. J. Sorensen, *J. Am. Chem. Soc.,* **2006**, *128*, 7025–7035.
[19] D. K. Fukushima, S. Dobriner and R. S. Rosenfeld, *J. Org. Chem.,* **1961**, *26*, 5025–5028.
[20] V. A. Vu, L. Bérillon and P. Knochel, *Tetrahedron Lett*., **2001**, *42*, 6847–6850.
[21] The compound **40** was synthesized according to the synthetic procedure reported by Li and Nan et al. However, some different results were observed. See the Supporting Information.
[22] A. L. Gemal and J-L Luche, *J. Am. Chem. Soc.*, **1981**, *103*, 5454–5459.
[23] A. Krief and D. Surleraux, *Synlett*, **1991**, *4*, 273–274.
[24] M. Leclaire and P. Jean, *Bull. Soc. Chim. Fr.*, **1996**, *133*, 801–803.
[25] A. Srikrishna, T. J. Reddy and P. P. Kumar, *Chem. Commun.*, **1996**, 1369–1370.
[26] E. Nakamura, S. Aoki, K. Sekiya, H. Oshiro and I. Kuwajima, *J. Am. Chem. Soc.* **1987**, *109*, 8056–8066.
[27] A.Sasaki, H. Matsumura, T. Yano, S. Takata, M. Sunagawa, *Chem. Pharm. Bull.* **1992**, *40*, 1098–1104.
[28] R. G-Fernandez, D. C-Tejeda, E. D. R-Nieto and F. A. Bermejo, *Tetrahedron,* **2010**, *66*, 8247–8253.
[29] A. F. Barrero, J. F. Q. del Moral, E. M. Sánchez and J. F. Arteaga, *Eur. J. Org. Chem.* **2006**, *7*, 1627–1641.
[30] a) A. Gansauer, T. Lauterbach, S. Narayan, *Angew. Chem. Int. Ed.,* **2003**, *42*, 5556–5573; b) J. Justicia, J. E. Oltra, A. F. Barrero, A. Guadano, A. Gonzalez-Coloma and J. M. Cuerva, *Eur. J. Org. Chem.,* **2005**, *4*, 712–718.
[31] The 3D structures and their structural homology of SOAT1 and SOAT2 have not yet been clarified because they are membrane proteins. Additionally, it is extremely difficult to isolate and crystallize.
[32] E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.,* **1959**, *37*, 911–917.

Entry for the Table of Contents



New A-ring simplified pyripyropene A analogs were synthesized by application of our total synthesis. Among the analogs, two analogs **7** and **10** exhibited the same efficacy for the SOAT2 inhibitory activity compared with that of natural pyripyropene A and extremely high isozyme selectivity as synthetic compounds. The analogs **7** and **10** were considered to be the most potent and selective synthetic SOAT2 inhibitor.