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ORIGINAL PAPER

Synthesis and conformational analysis of a potentially super-armed glucuronidation donor

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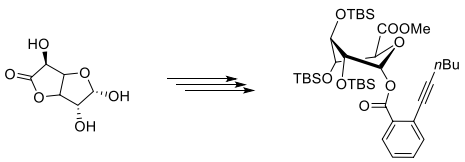
Abstract

The concise synthesis of a potentially "super-armed" glucuronidation donor is reported. The α-anomer was crystallized and analyzed by single crystal X-ray diffraction. The pyranose ring was found to be in a twist-boat conformation in the solid state. To confirm the relevance of this finding for the solution state, and explain the failure of analysis by NMR, DFT calculations were performed. They revealed the twist-boat to be the dominant among a group of several possible conformers at ambient temperature.

Graphical abstract



Keywords Carbohydrates · Crystal structure · Conformation · Protecting groups

Introduction

The efficient and stereoselective synthesis of glycosides and oligosaccharides is an old problem in synthetic organic chemistry [1]. The great advances in the field made over the last 50 years have informed the rapid progress of carbohydrate chemistry and glycobiology in the twenty-first century [2, 3]. However, there still exists no general and dependable methodology for glycosylation of arbitrary aglycones.

In memoriam: Prof. Fritz Sauter.

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Glucuronidation

Glucuronidation can be seen as a special case of glycosylation and is needed for the synthesis of mammalian secondary metabolites and a wide range of oligosaccharides [4]. It remains a particularly challenging task due to the comparably low reactivity of glucuronidation donors [5].

In eukaryotic cells, metabolic glucuronides are always in β-configuration. To achieve this, anchimeric assistance by ester groups at C-2 is typically employed. This approach frequently leads to orthoester formation and although many alternative protecting groups for C-2 exist, their introduction often requires long synthetic pathways [6].

There exist two strategies for glucuronidation: direct glucuronidation or glycosylation followed by oxidation. While the glycosylation-oxidation approach is more reliable in most cases, the substrate must be compatible with the final oxidation step (typically TEMPO/NaOCl). The development of effective direct glucuronidation donors is therefore needed for sensitive substrates.

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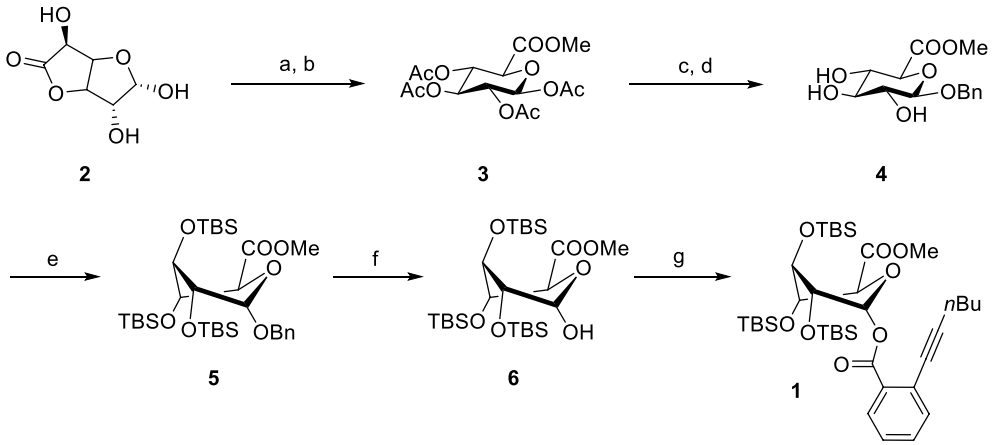
Scheme 1



Sch. 1 (a) NaOH, MeOH; (b) Ac_2O, py; (c) BnOH, TfOH, DCM; (d) NaOMe, MeOH; (e) TBSOTf, lutidine, DMF; (f) Pd/C, H_2, EtOH; (g) abzOH, DMAP, DCC, Et_3N, DCM

Yu glycosylation

Yu and coworkers have reported an effective and selective glycosylation methodology based on *ortho*-alkynylbenzoate (abz)—donors that are activated by catalytic amounts of gold[I]-triflates or -triflimides [7]. The group has since demonstrated the utility of their method on a diverse array of sensitive substrates, where standard glycosylation methodology like the Schmidt glycosylation failed [8].

"Super-armed"-concept

Studies of Fraser-Raid have shown the strong influence of protecting group strategy on donor reactivity and led to the introduction of the "armed/disarmed" concept [9]. The transition state in glycosylation reactions involves an electron-poor oxocarbenium-ion. Electron-withdrawing acetyl protection therefore further destabilizes the transition state and leads to less reactive "disarmed" donor molecules. In contrast, protection with (benzyl-) ethers does not withdraw electron density and leads to more reactive "armed" donors. In recent years, Bols and coworkers have extended this concept with the introduction of conformationally "super-armed" and "super-disarmed" donors. It was found that protection with bulky silyl ethers leads to a further increase in reactivity compared to benzyl ethers [10]. In super-armed donors, the pyranose ring is flipped from an equatorial-rich to an axial-rich conformation because of steric crowding of the equatorial plane. It was shown that the "super-arming" effect of silyl protection stems from this conformational change and not from inductive or silicon beta effects [11].

In combining the *ortho*-alkynyl benzoate leaving group with a super-armed silyl-protected glucuronic acid, we envisioned to create a very reactive glucuronidation donor without potential for the typical side reactions. The β-selectivity was to be provided by steric shielding from the bulky TBS (*tert*-butyldimethylsilyl) group at C-2.

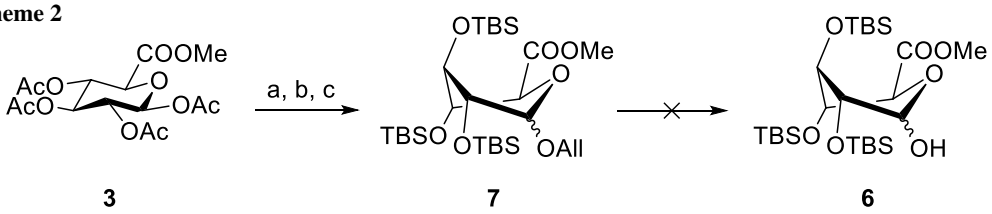
Results and discussion

The synthesis of donor **1** (Scheme 1) started by transforming glucuronolactone (**2**) into methyl 1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate (**3**) via known methods.

Further progress required the selective protection of C-1 with an easily cleavable protecting group that would withstand NaOMe in MeOH, which was used for acetyl deprotection. The allyl group was chosen and introduced via BF_3—promoted Fischer-glycosylation to circumvent the use of hyperstoichiometric amounts of silver salts in the traditional Koenigs-Knorr method. However, the anomeric allyl group in 7 unexpectedly could not be removed successfully (Scheme 2). Common methods like $Pd(PPh_3)_4$, Pd/C, $Rh(PPh_3)_4$, *t*-BuOK failed to perform the necessary isomerization, rendering the allyl group inert to cleavage.

The choice of protecting group was changed to benzyl, which was introduced into the molecule by Fischer-glycosylation. After installation of benzyl, the acetyl groups were cleaved with NaOMe in MeOH and the now free hydroxyl groups silylated with TBSOTf/lutidine. The hydroxyl groups at C2 and C4 were protected rapidly at ambient temperature while the subsequent reaction at C3 required gentle heating for 24 h. Anomeric benzyl was cleaved with hydrogen over Pd/C in EtOH overnight. Finally, the abz—group was

Scheme 2



3 7 6

Sch. 2 (a) AllOH, BF_3. Et_2O, DCM; (b) NaOMe, MeOH; (c) TBSOTf, lutidine, DMF

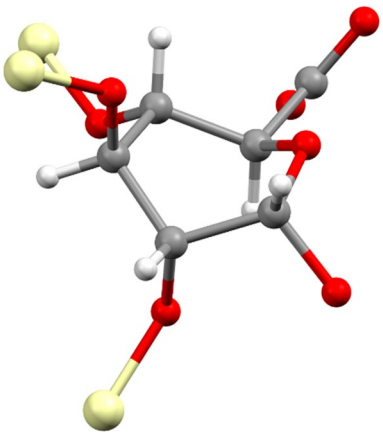


Fig. 1 The molecular structure of **1** in the solid state. Residues of the silyl protecting groups and the anomeric leaving group are omitted for clarity. The pyranose ring is distorted into a twist-boat conformation

synthesized according to known methods [12] and introduced via modified Steglich-esterification with DMAP/EDCI [7]. The reaction worked and gave the two anomers of the finished donor in a 3:2 (α:β) ratio.

Crystallography

The α-anomer **1** was successfully crystallized and the structure resolved by single crystal diffraction (Fig. 1). The pyranose ring can be described as an intermediate between a boat and a twisted boat conformation [13] with puckering parameters [14] of $Q = 0.766(3)$ Å, $\Theta = 87.9(2)°$, and $\varphi = 315.13(19)°$. Due to the absence of classical hydrogen-bonding donor groups, only weak non-classical intermolecular hydrogen bonds are observed in the packing of the molecules, here between a C–H group of the phenyl ring (C3–H3) and an O atom of the methyl ester moiety (O5) [H3···O5 = 2.58 Å, C3···O5 = 3.495(3) Å, C3–H3···O5 = 162°], as well as between a methyl group (C20–H20B) and the O atom of the pyranose ring (O3) [H20B···O3 = 2.51, C20···O3 = 3.270(4), C20–H20B···O3 = 135°].

The crystal structure confirms what was previously reported by Bols for his superarmed donors [10]: the structure is not merely flipped from a $^4C_1$ to a $^1C_4$ conformation, where all the substituents would be oriented axially, but is further distorted into a twist boat in order to better accommodate the bulky silyl groups. As the flat abz-group at the anomeric carbon and the methyl ester at C–6 are not sterically demanding and therefore not expected to further distort the molecule, it is not surprising that glucuronide **1** is conformationally similar to previously reported super-armed glycosyl donors.

Computational

To investigate the behavior of compound **1** in liquid phase solution and verify the relevance of the crystal structure, conformational analysis by ^{1}H NMR was attempted. However, no conclusive results could be obtained. To explain this, a DFT (Density Functional Theory) study was performed. Sixty-one possible conformers were found and analyzed. A Boltzmann weighing of these conformers showed that twenty have a contribution of at least 1%. These twenty conformers make up 89% of the ensemble and were investigated further. They can be grouped in three distinct groups based on their ring conformation. The greatest contribution of 66% can be attributed to a group of 12 conformers that show a twist-boat conformation (Fig. 2a) like the one observed in the crystal structure. This group also contains the lowest energy conformer (relative free energy = 0.00 kJ/mol). The second group of seven conformers makes up a total of 18% and shows a different twist-boat conformer. The lowest energy conformer of this group is at + 3.93 kJ/mol (Fig. 2b). A single conformer in a $^1C_4$ chair conformation was found with a relative free energy of + 0.42 kJ/mol accounting for 16% (Fig. 2c). This shows that three different ring conformations are accessible in chloroform solution with the most prominent form resembling the crystal structure, therefore, validating the result of the crystallographic study, but also explaining the failure of NMR analysis. Because NMR operates at a far longer timescale than the interconversion of the different families of conformers, analysis by NMR gives a virtual compound that represents a weighted average of the different conformers but has no physical meaning.

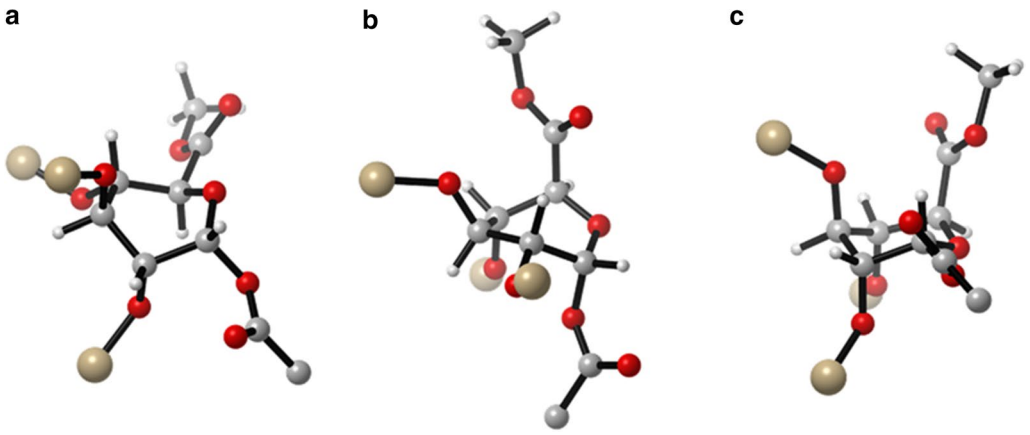


Fig. 2 Ring conformers for conformer group **a** 1, **b** 2, and **c** 3. The lowest energy conformer for each group is shown. Residues of the silyl protecting groups and the anomeric leaving group are omitted for clarity

Conclusion

In the course of this work, a glucuronidation donor with a distorted pyranose ring was synthesized and crystallized. Single-crystal X-ray diffraction showed the molecule to be in a conformation resembling a twist boat in solid phase. In-silico analysis suggested the molecule to exist as a mixture of different conformer families in solution, a twist-boat resembling the crystal structure being the most prominent.

Experimental

HPLC-grade solvents (acetonitrile, methanol) were sourced from VWR. All other non-specified chemicals were sourced from Sigma-Aldrich. Moisture- and air-sensitive reactions were carried out in flame-dried glass vessels under an argon atmosphere using Schlenk techniques. Chromatography was performed with glass columns and Merck silica gel 43–60 µm. TLC (Thin Layer Chromatography) analysis was performed with pre-coated aluminum-backed plates (silica gel 60 F254, Merck). Compounds were visualized by submerging in *p*-anisaldehyde/ethanol and drying with a hot air gun. NMR spectra were recorded on a Bruker Avance 400 at 400 MHz (101 MHz). Chemical shifts are given in ppm and were referenced to the solvent residual peaks. Coupling constants are given in Hertz. Specific rotations were measured on an Anton Paar MCP 500 polarimeter at 20 °C and 589 nm. HR-MS measurements were carried out in acetonitrile, methanol, water or a mixture on an Agilent 1100/1200 HPLC with binary pumps, a degasser and a column thermostat and an Agilent 6230 AJS ESI-TOF mass spectrometer. Data analysis was carried out using MassHunter Qualitative Analysis software (Agilent).

Methyl 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronate (2) Glucuronic acid γ-lactone (17.6 g, 99.9 mmol, 1.00 eq) was suspended in 100 cm^3 dry MeOH and 0.01 cm^3 of a 5 M NaOMe solution (2.70 mg, 0.10 mmol, 0.01 eq) were added under vigorous stirring. After 80 min, the solution was neutralized with formic acid and the solvent was removed under reduced pressure, giving a white foam. The foam was dissolved in 40 cm^3 dry pyridine (39.5 g, 499 mmol, 5.00 eq) and 1.22 g DMAP (9.99 mmol, 0.10 eq) was added. Acetyl chloride (32.0 cm^3, 449 mmol, 4.50 eq) was added at − 40 °C over the course of one hour and the reaction mixture was then warmed up to room temperature.

The reaction mixture was washed with sat. aq. $NaHCO_3$ solution, 1 M HCl, water and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the brown residue was dissolved in 80 cm^3 of ethyl acetate and stored at − 20 °C overnight to induce crystallization. After filtration, white crystals (10.2 g, 27%) of pure *β*-anomer were obtained. Yield: 10.20 g (27%); $R_f = 0.70$ (PE/EA = 3:1); ^{1}H NMR is in accordance with literature [15].

Methyl 1-*O*-benzyl-2,3,4-tri-*O*-acetyl-*α*/*β*-D-glucopyranuronate (3) Substance **2** (8.64 g, 23.0 mmol, 1.00 eq) and 12.0 cm^3 of benzyl alcohol (115 mmol, 5.00 eq) were dissolved in 120 cm^3 dry DCM and 0.41 cm^3 of triflic acid (4.59 mmol, 0.20 eq) were added dropwise at 0 °C. The mixture was stirred overnight.

When TLC indicated complete consumption of starting material, the reaction was quenched with 20 cm^3 of 1 M aq. K_2CO_3 and washed with water and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. After evaporation of the solvent, 17.9 g of an amber oil were obtained. The oil contained residual benzyl alcohol and was used without further purification for the next step.

For analytical purposes, a small sample of the crude material was purified via column chromatography (20 g silica gel, petroleum ether/ethyl acetate = PE/EA = 5:1), obtaining-pure 3 with ^{1}H NMR identical to prior reports [16]. Yield: 17.86 g (crude oil); R_f = 0.49 (PE:EA = 1:1).

Methyl 1-*O*-benzyl-*α/β*-D-glucopyranuronate (4) Crude **3** (16.3 g, 38.5 mmol, 1.00 eq) was dissolved in 300 cm^3 MeOH and 0.77 cm^3 of 5 M NaOMe solution (3.85 mmol, 0.1 eq) were added dropwise at 0 °C. After 1 h, TLC indicated complete deprotection and the solution was quenched with formic acid and evaporated. The resulting crude oil (15.3 g) was purified via flash column chromatography (150 g silica gel, PE/EA = 1:1, then EA/MeOH = 10:1), yielding 3.2 g of a yellow foam. Analytical data were in accordance to literature precedent [16]. Yield: 3.20 g (47% over two steps); R_f = 0.23 (100% EA).

Methyl 1-*O*-benzyl-2,3,4-tri-*O*-(*tert*-butyldimethysilyl)-*α/β*-D-glucopyranuronate (5) Substance **4** (1.02 g, 3.43 mmol, 1.00 eq) was dissolved in 40 cm^3 dry DCM and 3.60 cm^3 2,6-lutidine (30.9 mmol, 9.00 eq) and 4.70 cm^3 TBSOTf (5.44 g, 20.6 mmol, 6.00 eq) were added dropwise at 0 °C and then stirred for 1 h. The reaction mixture was heated to reflux and stirred for 6 h.

When TLC indicated complete conversion, the solution was diluted with 50 cm^3 DCM and washed with 1 M HCl, sat. aq. $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product (2.42 g) was purified via column chromatography (40 g silica gel, PE/EA = 10:1), yielding 2.19 g of a yellow oil. ^{1}H NMR is in accordance with literature [16]. Yield: 2.19 g (99%); R_f = 0.64 (PE/EA = 10:1).

Methyl 2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-D-glucopyranuronate (6, $C_{25}H_{54}O_7Si_3$) Substance **5** (1.98 g, 2.96 mmol, 1.00 eq) was dissolved in 30 cm^3 EtOH and Pd/C (0.59 g, 5 w %) was added. The suspension was stirred under H_2 atmosphere overnight and filtered over a short plug of Celite. After evaporation of volatiles, 1.63 g of the crude residue was isolated. Purification was performed by column chromatography (100 g silica gel, PE/EA = 20:1), yielding 1.45 g of a colorless oil. An analytical sample of the *β*-anomer could be isolated from the anomeric mixture by repeated column chromatography (PE/EA = 20:1). Yield: 1.45 g (85%); R_f = 0.42 (PE/EA = 10:1); $[\alpha]^{20}_D$ = + 16.8° (*c* = 1 g/100 cm^3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.38 (dd, *J* = 12.61, 3.03 Hz, 1H), 4.48 (dd, *J* = 6.29, 2.49 Hz, 1H), 4.17–4.07 (m, 1H), 3.92–3.84 (m, 1H), 3.75 (d, *J* = 6.79 Hz, 3H), 3.69–3.62 (m, 1H), 0.97–0.83 (m, 27H), 0.16–0.04 (m, 18H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 89.2, 76.5, 73.5, 71.5, 71.3, 52.2, 26.2–25.7 ppm; HRMS (ESI): *m/z* calcd. for $C_{25}H_{54}O_7Si_3$ ($[M + Na]^+$) 573.3075, found 573.3097 (+ 5.0 ppm).

Methyl 1-*O*-[2-(hex-1-inyl)benzoyl]-2,3,4-tri-*O*-(*tert*-butyl-dimethylsilyl)-*α*-D-glucopyranuronate (1, $C_{38}H_{66}O_8Si_3$) Substance **6** (1.00 g, 2.66 mmol) was dissolved in 25 cm^3 dry DCM and 1.11 cm^3 Et_3N (0.81 g, 7.97 mmol, 3 eq), 0.42 g DMAP (3.45 mmol, 1.3 eq) and 0.66 g EDCI (3.45 mmol, 3 eq) were added at room temperature and stirred for 10 min. 2-(1-Hexynyl)benzoic acid (0.64 g, 3.19 mmol, 1.2 eq) was added dropwise and the mixture was stirred overnight. The mixture was heated to 40 °C and stirred for another 3 h. After cooling down to ambient temperature, the reaction mixture was diluted with 100 cm^3 DCM and washed with 1 M HCl, sat. aq. $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and volatiles were removed in vacuo. The residue was purified by column chromatography (100 g silica gel, PE/EA = 20:1). After purification, a mixture of anomers (452 mg) was obtained and purified again via column chromatography (40 g silica gel, PE/EA = 20:1). Separation of anomers was not possible, and 412 mg of a 3:2 anomeric mixture of *α/β*-**1** was obtained. Crystals were grown by preparing a 2% solution of the material in methanol, to which water was added until the solution became turbid. An oil separated after few minutes. Acetone was added dropwise under constant shaking until the solution became almost clear again and the oil redissolved. After storing this mixture at 4 °C overnight, colorless crystals of pure *α*-anomer could be harvested. Yield: 452 mg (23%); R_f = 0.29 (PE:EA = 3:1).

α–Anomer: m.p.: 81.6–82.7 °C; $[\alpha]^{20}_D$ = + 46.6° (*c* = 1 g/100 cm^3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (dd, *J* = 8.0, 0.9 Hz, 1H), 7.50 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.41 (td, *J* = 7.6, 1.4 Hz, 1H), 7.31–7.24 (m, 1H), 6.47 (d, *J* = 2.9 Hz, 1H), 4.69 (d, *J* = 5.8 Hz, 1H), 4.11 (ddd, *J* = 5.9, 2.0, 0.9 Hz, 1H), 4,07 (ddd, *J* = 4.0, 2.9, 0.9 Hz, 1H), 3.95 (dd, *J* = 4.2, 1.9 Hz, 1H), 3.76 (s, 3H), 2.48 (d, *J* = 7.2 Hz, 3H), 1.67–1.54 (m, 2H), 1.54–1.44 (m, 2H), 0.9–0.80 (m, 27H), 0.18–−0.06 (m, 18H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.3, 164.2, 134.2, 131.7, 131.4, 130.7, 126.8, 125.2, 96.6, 91.6, 79.2, 76.5, 75.1, 73.4, 69.9, 52.1, 30.6, 26.0, 25.9, 25.8, 22.0, 19.5, 18.2, 18.1, 17.9, 13.7, −4.1, −4.3, −4.4, −4.5, −4.9, −5.0 ppm; HRMS (ESI): *m/z* calcd. for $C_{38}H_{67}O_6Si_3$ ($[M + H]^+$) 735.4138, found 735.4097 (−5.6 ppm).

***β*-Anomer (analytical data from enriched mother liquor after crystallization)** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.31 (t, *J* = 7.7 Hz, 1H), 6.25 (d, *J* = 6.2 Hz, 1H), 4.58 (d, *J* = 1.6 Hz, 1H), 4.36 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.97 (d, *J* = 6.3 Hz, 1H), 3.84 (d, *J* = 3.5 Hz, 1H), 3.69 (s, 3H), 2.50 (t, *J* = 7.0 Hz, 2H), 1.66–1.45 (m, 6H), 1.00–0.84 (m, 27H),

0.17 − 0.05 (m, 18H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.9, 164.0, 134.4, 131.8, 130.9, 130.8, 126.7, 125.6, 96.9, 93.6, 79.3, 79.1, 77.4, 75.0, 72.6, 52.2, 30.7, 25.8, 25.7, 25.7, 22.0, 19.6, 18.0, 13.7, − 4.4, − 4.6, − 4.6, − 4.8, − 4.9 p pm; HRMS (ESI): *m/z* calcd. for $C_{38}H_{67}O_6Si_3$ ($[M+H]^+$) 735.4138, found 735.4102 (− 5.0 ppm).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00706-022-03009-4.

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Data availability No data is available.

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