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Synthesis of Biotinylated Galiellalactone Analogues

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Abstract

Two biotinylated derivatives of the fungal metabolite galiellalactone (1) were synthesized in order to facilitate the investigation of the molecular mechanism of action of the galiellalactonoids. Galiellalactone is a STAT3-signaling inhibitor that inhibits growth *in vitro* as well as *in vivo* of prostate cancer cells expressing activated STAT3. To provide a suitable point of attachment for biotin, the 8-hydroxymethyl derivative (3) and its 7-phenyl analogue 4 were synthesized by a modified tandem Pd-catalysed carbonylation and intramolecular vinyl allene Diels-Alder procedure previously developed. The two primary alcohols obtained, 3 and 4, were coupled to biotin as the 6-aminohexanoic acid amide, activated as the acid chloride, yielding the derivatives 5 and 6.

Keywords

STAT3, Galiellalactone, Biotin, Synthesis

1. Introduction

The protein STAT3 (Signal Transducer and Activator of Transcription 3) is a transcription factor that is involved in different cellular processes. It has been shown to be constitutively activated in malignancies and is involved in the proliferation of several types of cancer cells [1] [2]. In normal cells, the activation of STAT3 must consequently be tightly regulated, and STAT3 is today considered to be a relevant target for novel drugs for the treatment of cancer [3]. Galiellalactone (**1**) is a fungal metabolite that inhibits the STAT3 signaling pathway, presumably by reacting covalently with the thiol group of critical cysteines of STAT3 and thereby blocking the

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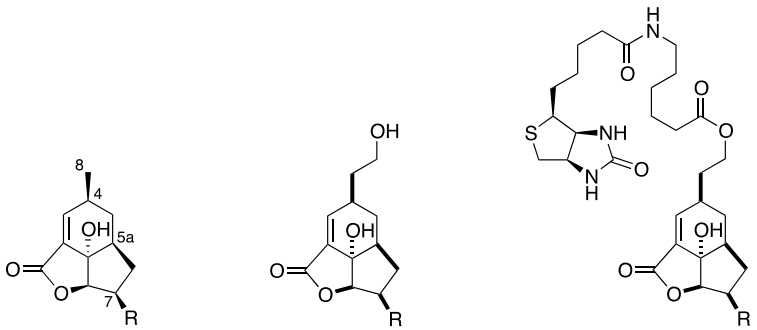
binding of STAT3 to STAT3 specific transcriptional DNA elements [4]. **1** inhibits the growth, both *in vitro* and *in vivo*, of prostate cancer cells expressing activated STAT3 and inhibits the expression of STAT3 regulated genes and proteins, including anti-apoptotic genes [4]. Furthermore, galiellalactone (**1**) inhibits growth and induces apoptosis of prostate cancer stem cell-like cells expressing phosphorylated STAT3 (pSTAT3) [5], and has the potential to be developed into a drug for treating cancers in which STAT3 is activated.

1 contains an α,β-unsaturated lactone moiety, and it has been demonstrated to react with sulfur nucleophiles to produce inactive adducts [6] [7]. It has been proposed that **1** interferes directly with the binding of STAT3 to DNA by reacting with a thiol group of a cysteine in STAT3 that is located in a domain of STAT3 that is involved in the binding to DNA [4], however, no evidence supporting this molecular mechanism of action has yet been presented. Target identification and mechanism of action elucidation are highly challenging yet crucial areas of chemistry and biology, and several methods and strategies have been developed to achieve this. Compounds that covalently react with their target provide an advantage as they can be modified or tagged with moieties that allow for the detection or isolation of the compound-target adducts. Biotin labeling of covalent inhibitors is an effective method for detecting and isolating bound target proteins because of the high affinity biotin displays to streptavidin allowing the capture and detection of biotin labeled inhibitor-target adducts. A main challenge is to attach the biotin label to the inhibitor in such a way that the target affinity is not abolished. We envisioned that a hydroxymethyl group in position 8 of **1** (see **Figure 1**) would allow us to attach a biotin group via a suitable linker so that the STAT3 inhibiting effect was retained. To this end, 8-hydroxymethyl-galiellalactone (**3**) as well as 8-hydroxymethyl-7-phenyl galiellalactone (**4**) was prepared by a modified tandem Pd-catalysed carbonylation and intramolecular vinyl allene Diels-Alder strategy previously developed for the synthesis of 4-*epi*-**1** [8]. **3** and **4** were then coupled with an ester bond to the 6-aminohexanoic acid amide of biotin, yielding the two desired compounds **5** and **6** (**Figure 1**). Both enantiomers of galiellalactone (**1**) have been prepared [9] [10] and found to be an equally potent inhibitors of IL-6 mediated STAT3 signaling [7] [11], consequently **3** and **4** were prepared as racemates while **5** and **6** were obtained as pairs of diastereomers. During our work with analogues of **1** [8], we had indications (unpublished results) that a substituent in position 7 was beneficial for the potency, and to investigate if a phenyl group at C-7 affects the STAT3 inhibiting effect of galiellalactone (**1**) the 7-phenyl analogue **2** was prepared and assayed.

2. Experimental Section

Reagents and solvents were used from commercial sources without purification, except THF and CH_2Cl_2 that were passed through a MBraun SPS-800 solvent system. All reactions were carried out in standard dry glassware and atmospheric surroundings. Analytical thin layer chromatography (TLC) was performed on Kiselgel 60 F254 plates (Merck) and visualized by spraying with vanillin/H_2SO_4 and heating. Silica gel column chromatography was performed on SiO_2 (Matrex LC-gel: 60A, 35-70 MY, Grace). ^1H and ^{13}C NMR spectra were rec-



1 R = H
2 R = Ph

3 R = H
4 R = Ph

5 R = H
6 R = Ph

Figure 1. Structures of the fungal metabolite galiellalactone (**1**), (+)-7-phenyl galiellalactone (**2**), the two analogues 8-hydroxymethyl galiellalactone (**3**) and 8-hydroxymethyl-7-phenyl galiellalactone (**4**), and the corresponding *N*-(+)-biotinyl-6-aminohexanoic acid esters **5** and **6**.

orded with a Bruker DRX 400 spectrometer (at 400 MHz for ^{1}H and 100 MHz for ^{13}C) and a Bruker DRX 500 spectrometer (at 500 and 125 MHz, respectively). The spectra were recorded in $CDCl_3$ and CD_3OD, and the solvent signals (7.27/77.0 and 3.31/49.0 ppm) were used as reference. The data for the ^{1}H signals are given as chemical shifts in ppm and (number of protons, multiplicity, and coupling constants (*J*) in Hz), while the ^{13}C data are given as chemical shifts in ppm. HR-ESIMS spectra were recorded with a Waters Q-TOF Micro system.

Hept-6-en-1-yn-3-ol(**8a**): To a solution of ethynyl magnesium bromide (100 ml, 0.5 M in THF, 50 mmol) was slowly added to a solution of 4-pentenal (**7a**, 3.70 g, 44.0 mmol) in 54 ml of dried THF at 0˚C. After the addition was complete, the solution was allowed to reach room temperature and stirred overnight. The reaction mixture was quenched by the addition of 50 ml NH_4Cl (sat.), the mixture was extracted 3 times with 80 ml EtOAc and the organic phase was dried with $MgSO_4$ and concentrated under reduced pressure. **8a** was obtainedas a yellowish oil (4.75 g, quantitative yield), which was used without further purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) 5.69 (1 H, ddt, 17.1, 10.2, 6.7), 4.93 (1 H, ddd, 17.1, 3.4, 1.5), 4.85 (1 H, ddd, 10.2, 3.4, 1.5), 4.25 (1 H, td, 6.7, 2.1), 2.37 (1 H, d, 2.1), 2.10 (2 H, m), 1.67 (2 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 137.5, 115.4, 84.6, 73.2, 61.7, 36.6, 29.2. HRMS calcd for $C_7H_{11}O$ [M + H]: 111.0810, found: 111.0834.

4-Phenylhept-6-en-1-yn-3-ol(**8b**): Phenylacetic acid (30.0 g, 220 mmol) was dissolved in 100 ml MeOH and $SOCl_2$ (28.8 g, 242 mmol) was added dropwise at 0˚C under stirring. The reaction mixture was allowed to reach room temperature overnight and the volatiles were removed under reduced pressure. Phenylacetic acid methyl ester was obtained as a yellowish oil (32.8 g, quantitative yield) and used directly in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) 7.34 (5 H, m), 3.72 (3 H, s), 3.67 (2 H, s). ^{13}C NMR (100 MHz, $CDCl_3$) 171.0, 133.6, 128.6, 127.8, 126.3, 51.0, 40.2. HRMS calcd for $C_9H_{10}O_2Na$ [M + Na]: 173.0578, found: 173.0558. Phenylacetic acid methyl ester (14.0 g, 93 mmol) was dissolved in 150 ml dry THF and added slowly at −78˚C over 30 min to a freshly prepared LDA solution [12] 3 g, 120 mmol, diisopropyl amine and 67 ml, 107 mmol, of n-BuLi (1.6 M in hexane)]. The reaction mixture was stirred for 30 min at −78˚C before allyl bromide (17.0 g, 141 mmol) was added dropwise at −78˚C over 10 min. The reaction mixture was quenched by the addition of 120 ml NH_4Cl (sat.), extracted 3 times with 100 ml EtOAc, the combined organic phases were dried ($MgSO_4$) and concentrated by evaporation to afford methyl 2-phenylpent-4-enoate as a yellowish oil (17.7 g, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) 7.27 (3 H, m), 7.22 (2 H, m), 5.68 (1 H, ddt, 17.0, 10.2, 6.8), 5.04 (1 H, ddd, 17.0, 2.9, 1.3), 4.96 (1 H, ddt, 10.2, 2.9, 1.3), 3.61 (1 H, t, 7.8), 3.61 (3 H, s), 2.80 (1 H, m), 2.48 (1 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 173.8, 138.5, 135.2, 128.6, 127.8, 127.3, 116.9, 51.9, 51.3, 37.5. HRMS calcd for $C_{12}H_{14}O_2Na$ [M + Na]: 213.0891, found: 213.0891. Methyl 2-phenylpent-4-enoate (8.7 g, 46 mmol) in 200 ml of dry CH_2Cl_2 was reduced to **7b** by slowly adding DIBAL-H (48.0 ml, 1 M in hexane, 48.0 mmol) under N_2 atmosphere at -78˚C and stirring for 3 h at −78˚C. Without work-up and purification, ethynyl magnesium bromide (100 ml, 0.5 M in THF, 50 mmol) was added dropwise to the reaction mixture which was stirred for an additional 30 min at −78˚C and left at room temperature overnight before the reaction was quenched by the addition of 120 ml $NaHCO_3$ (sat.). The mixture was extracted 4 times with 80 ml EtOAc and the combined organic phases were dried with $MgSO_4$ and concentrated by evaporation. The crude product was purified by silica gel chromatography, and **8b** (3.5 g, 41%) was obtained as a 1:0.2 mixture of diastereomers. *Diastereomer* a) ^{1}H NMR (400 MHz, $CDCl_3$) 7.24 (5 H, m), 5.64 (1 H, ddd, 17.0; 10.1, 6.2), 5.03 (1 H, ddd, 17.0, 3.1, 1.3), 4.93 (1 H, ddt, 10.1, 3.1, 1.3), 4.48 (1 H, dd, 8.5, 2.2), 2.94 (1 H, dt, 8.5, 6.2), 2.65 (1 H, m), 2.52 (1 H, m), 2.39 (1 H, d, J 2.2). ^{13}C NMR (100 MHz, $CDCl_3$) 139.1, 135.8, 129.1, 128.0, 127.0, 116.6, 82.8, 74.9, 65.3, 50.8, 35.2. *Diastereomer* b) ^{1}H NMR (400 MHz, $CDCl_3$) 7.24 (5 H, m), 5.64 (1 H, ddd, 17.0; 10.1, 6.2), 5.03 (1 H, ddd, 17.0, 3.1, 1.3), 4.93 (1 H, ddt, 10.1, 3.1, 1.3), 4.48 (1 H, ddd, 8.5, 2.2), 2.92 (1 H, dt, 8.5, 6.2), 2.67 (1 H, m), 2.52 (1 H, m), 2.43 (1 H, d, 2.2). ^{13}C NMR (100 MHz, $CDCl_3$) 139.2, 135.9, 128.8, 128.2, 126.9, 116.7, 83.2, 74.6, 65.5, 51.4, 35.1. HRMS calcd for $C_{13}H_{14}ONa$ [M + Na]: 209.0942, found: 209.0919.

(*Z*)-11-((Triisopropylsilyl)oxy)undeca-1,8-dien-6-yn-5-ol(**10a**): TEA (55 ml, 400 mmol) was added to a mixture of $PdCl_2(PPh_3)_2$ (0.70 g, 0.99 mmol) and CuI (0.38 g, 1.99 mmol) at 0˚C under nitrogen atmosphere and the solution was stirred for 30 minutes. To this solution **9** (7.25 g, 20.5 mmol), prepared according to reference 12 and identical in all aspects with the reported compound, in 40 ml dry THF was added dropwise followed by **8a (**2.19 g, 20.0 mmol) in 40 ml dry THF at 0˚C. The reaction mixture was stirred overnight at room temperature, quenched with 50 ml $NaHCO_3$ (sat.), extracted 3 times with 60 ml EtOAc. The combined organic phases were dried with Na_2SO_4 and concentrated by evaporation. The yield was quantitative and **10a** was used directly in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) 6.04 (1 H, dt, 10.8, 7.0), 5.85 (1 H, ddt, 17.0, 10.2, 7.2), 5.56 (1 H, ddd, 10.8, 3.1, 1.4), 5.08 (1 H, ddd, 17.0, 3.2, 1.5), 5.00 (1 H, dd, 10.2, 3.2), 4.54 (1 H, td, 6.5,

3.1), 3.76 (2 H, t, 7.0), 2.56 (2 H, qd, J 7.0, 1.4), 2.26 (2 H, td, J 7.2, 1.5), 1.83 (2 H, m), 1.13 - 1.03 (21 H, m).^{13}C NMR (100 MHz, $CDCl_3$) 141.1, 137.9, 115.5, 110.0, 94.4, 82.1, 62.7, 62.5, 37.1, 34.4, 29.7, 18.2, 12.2. HRMS calcd for $C_{20}H_{37}O_2Si$ [M + H]: 337.2563, found: 337.2560.

(*Z*)-Methyl (11-((triisopropylsilyl)oxy)undeca-1,8-dien-6-yn-5-yl) carbonate(**11a**): **10a** (6.70 g, 20.0 mmol) was dissolved in 130 ml of dry CH_2Cl_2 at 0˚C. DMAP (4.86 g, 39.8 mmol) was added and the mixture was stirred for 20 min. Methyl chloroformate (5.6 g, 59.7 mmol) was added dropwise, and the reaction was brought to room temperature overnight before 50 ml $NaHCO_3$ (sat.) was added and the mixture was extracted 2 times with 80 ml CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography with CH_2Cl_2/heptane 1:1 as eluent, giving 3.59 g (46%) of **11a** as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) 6.09 (1 H, dt, 10.8, 7.3), 5.82 (1 H, ddt, 17.0, 10.2, 6.6), 5.56 (1 H, ddd, 10.8, 3.3, 1.7), 5.40 (1 H, td, 6.6, 1.7), 5.07 (1 H, ddd, 17.0, 3.2, 1.3), 5.02 (1 H, ddd, 10.2, 3.2, 1.3), 3.81 (3 H, s), 3.76 (2 H, t, 6.5), 2.55 (2 H, qd, 6.6, 1.3), 2.25 (2 H, m), 1.95 (2 H, m), 1.09 - 1.03 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 155.2, 142.3, 137.0, 115.9, 109.6, 89.9, 83.7, 68.5, 62.5, 55.1, 34.4, 34.3, 29.4, 18.2, 12.2. HRMS calcd for $C_{22}H_{39}O_4NaSi$ [M + H]: 395.2618, found: 395.2647.

(*Z*)-4-Phenyl-11-((triisopropylsilyl)oxy)undeca-1,8-dien-6-yn-5-ol(**10b**):**10b** was prepared in the same way as **10a**, from **8b**, and **10b** was obtained in a quantitative yield as a 1:0.2 mixture of diastereomers. *Diastereomer* a) ^{1}H NMR (400 MHz, $CDCl_3$) 7.30 (5 H, m), 6.05 (1 H, dt, 10.8, 7.4), 5.71 (1 H, ddt, 17.1, 10.2, 6.3), 5.55 (1 H, ddd, 10.8, 3.3, 1.5), 5.08 (1 H, ddd, 17.1, 3.2, 1.6), 4.99 (1 H, ddd, 10.2, 3.2, 1.6), 4.74 (1 H, ddd, 7.5, 5.7, 1.5), 3.74 (2 H, t, 6.5), 3.04 (1 H, m), 2.65 (2 H m), 2.51 (2 H, m), 1.77 (1 H, d, 7.5), 1.17 - 1.04 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 141.2, 139.4, 136.1, 129.2, 128.2, 127.2, 116.7, 109.6, 92.4, 83.8, 66.4, 62.4, 51.4, 35.6, 34.1, 18.0, 12.0. *Diastereomer* b) ^{1}H NMR (400 MHz, $CDCl_3$) 7.30 (5 H, m), 6.03 (1 H, dt, 10.8, 7.4), 5.71 (1 H, ddt, 17.1, 10.2, 6.3), 5.57 (1 H, ddd, 10.8, 3.3, 1.5), 5.06 (1 H, ddd, 17.1, 3.2, 1.6), 4.99 (1 H, ddd, 10.2, 3.2, 1.6), 4.77 (1 H, ddd, 7.5, 5.7, 1.5), 3.78 (2 H, t, 6.5), 3.01 (1 H, m), 2.70 (2 H m), 2.51 (2 H, m), 1.78 (1 H, d, 7.5), 1.17 - 1.04 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 141.3, 139.6, 136.2, 128.9, 128.4 127.1, 116.8, 109.7, 92.8, 83.5, 66.5, 62.4, 52.1, 35.6, 34.2, 18.0, 12.0.HRMS calcd for $C_{26}H_{40}O_2NaSi$ [M + Na]: 435.2695, found: 435.2663.

(*Z*)-Methyl (4-phenyl-11-((triisopropylsilyl)oxy)undeca-1,8-dien-6-yn-5-yl) carbonate(**11b**):**11b** was prepared in the same way as **11a**, from **10b**, and **11b** was obtained in a 75% yield as a 1:0.2 mixture of diastereomers. *Diastereomer* a) ^{1}H NMR (400 MHz, $CDCl_3$) 7.28 (5 H, m), 6.04 (1 H, dt, 11.5, 6.9), 5.65 (1 H, ddd, 17.1, 10.9, 6.7), 5.58 (1 H, dd, 4.8, 2.1), 5.48 (1 H, ddd, 11.5, 2.1, 1.6), 5.04 (1 H, ddd, 17.1, 3.2, 1.5), 4.96 (1 H, ddd, 10.9, 3.2, 1.5), 3.78 (3 H, s), 3.68 (2 H, t, 6.4), 3.15 (1 H, m), 2.71 (1 H, m), 2.56 (1 H, m), 2.37 (2 H, dtd, 6.9, 6.4, 1.6), 1.17 - 0.95 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 154.9, 142.1, 139.2, 135.4, 128.8, 128.2, 127.1, 117.0, 109.2, 88.5, 84.6, 71.7, 62.3, 54.9, 49.4, 35.5, 34.0, 18.0, 11.9. *Diastereomer* b) ^{1}H NMR (400 MHz, $CDCl_3$) 7.28 (5 H, m), 6.06 (1 H, dt, 11.5, 6.9), 5.66 (1 H, ddd, 17.1, 10.9, 6.7), 5.58 (1 H, dd, 4.8, 2.1), 5.50 (1 H, ddd, 11.5, 2.1, 1.6), 5.05 (1 H, ddd, 17.1, 3.2, 1.5), 4.97 (1 H, ddd, 10.9, 3.2, 1.5), 3.76 (3 H, s), 3.70 (2 H, t, 6.4), 3.15 (1 H, m), 2.73 (1 H, m), 2.57 (1 H, m), 2.50 (2 H, dtd, 6.9, 6.4, 1.6), 1.17 - 0.95 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 154.8, 142.3, 138.9, 135.2, 128.8, 128.2, 127.1, 116.9, 109.3, 88.46, 84.7, 71.8, 62.3, 54.8, 49.7, 34.9, 34.1, 18.0, 11.9. HRMS calcd for $C_{28}H_{42}O_4NaSi$ [M + Na]: 493.2750, found: 493.2773.

rac-(6*R*,7a*R*)-Methyl 6-(2-((triisopropylsilyl)oxy)ethyl)-2,6,7,7a-tetrahydro-1*H*-indene-4-carboxylate(**12a**): To a dried autoclave flask was added $Pd(OAc)_2$ (0.41 g, 1.8 mmol) and DPPP (0.75 g, 1.8 mmol) under a nitrogen atmosphere, followed by 9 ml dry toluene and **11a** (3.59 g, 9.1 mmol) dissolved in 9 ml toluene/MeOH (1:1). CO was bubbled though the solution before the autoclave was pressurized with CO to 5 bar. The reaction mixture was stirred for 48 h at 5 bar at room temperature before being diluted with 15 ml EtOAc and filtered through a celite plug. The filtered solution was concentrated and purified by silica gel chromatography with heptane/Et_2O (94:6) to afford 0.55 g (16%) of **12a** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) 6.82 (1 H, d, 2.1), 6.27 (1 H, dd, 6.1, 2.6), 3.82 (2 H, m), 3.78 (3 H, s), 2.73 (1 H, m), 2.72 (1 H, dddd, 16.4, 8.8, 5.2, 2.1), 2.44 (2 H, ddd, 11.5, 4.5, 2.6), 2.14 (1 H, m), 2.12 (1 H, m), 1.77 (1 H, td, 13.5, 6.6), 1.60 (1 H, td, 13.5, 5.2), 1.37 (1 H, ddd, 12.5, 11.5, 3, 6.1), 1.10 (1 H, m), 1.08 - 1.04 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 167.2, 145.7, 137.8, 127.0, 126.9, 61.2, 51.7, 44.7, 38.6, 36.4, 35.2, 32.4, 31.2, 18.2, 12.2. HRMS calcd for $C_{22}H_{38}O_3NaSi$ [M + Na]: 401.2518, found: 401.2488.

Methyl 2-phenyl-6-(2-((triisopropylsilyl)oxy)ethyl)-2,6,7,7a-tetrahydro-1*H*-indene-4-carboxylate(**12b**):**12b** was prepared in the same way as **12a**, starting from **11b** (6.55 g, 13.9 mmol). **12b** was obtained as colourless oil as a 1:0.8 inseparable mixture of two diastereomers (total yield 2.64 g, 42%). *Diastereomer* a) ^{1}H NMR (400 MHz,

$CDCl_3$) 7.24 (5 H, m), 6.94 (1 H, t, 2.5), 6.32 (1 H, m), 4.02 (1 H, m), 3.85 (2 H, m), 3.79 (3 H, s), 2.86 (1 H, m), 2.75 (1 H, m), 2.60 (1 H, dt, 12.3, 7.0), 2.16 (1 H, dd, 10.2, 2.5), 2.00 (1 H, dt, 10.2, 9.2), 1.81 (1 H, tt, 13.2, 6.5), 1.65 (1 H, dt, 13.2, 6.5), 1.37 (1 H, dt, 12.3, 10.2), 1.21 - 0.91 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 166.9, 146.8, 146.0, 138.3, 130.4, 129.4, 128.4, 127.5, 126.6, 126.1, 60.9, 51.6, 51.3, 50.3, 44.6, 43.2, 42.2, 39.8, 38.4, 36.2, 35.2, 18.0, 12.0. *Diastereomer* b) ^{1}H NMR (400 MHz, $CDCl_3$) 7.24 (5 H, m), 6.40 (1 H, m), 6.32 (1 H, m), 4.02 (1 H, m), 3.85 (2 H, m), 3.78 (3 H, s), 2.96 (1 H, m), 2.75 (1 H, m), 2.60 (1 H, dt, 12.3, 7.0), 2.16 (1 H, dd, 10.2, 2.5), 2.00 (1 H, dt, 10.2, 9.2), 1.81 (1 H, tt, 13.2, 6.5), 1.61 (1 H, m), 1.37 (1 H, dt, 12.3, 10.2), 1.21 - 0.91 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 166.9, 146.7, 145.6, 138.4, 130.4, 129.3, 128.4, 127.2, 126.6, 126.0, 60.9, 51.6, 51.3, 50.3, 44.6, 43.2, 42.2, 39.8, 38.3, 36.1, 35.0, 18.0, 12.0. HRMS calcd for $C_{28}H_{43}O_3Si$ [M + H]: 455.2981, found: 455.2956.

rac-(1a*S*,3a*R*,5*R*,7a*S*)-Methyl 5-(2-((triisopropylsilyl)oxy)ethyl)-1a,2,3,3a,4,5-hexahydroindeno [1,7a-*b*]oxirene-7-carboxylate(**13a**): **12a** (0.55 g, 1.4 mmol) was dissolved in 30 ml dry CH_2Cl_2 under nitrogen atmosphere and *m*-CPBA (0.32 g, 1.9 mmol) was added at 0˚C. The reaction mixture was stirred at this temperature for 1 h before it was quenched by the addition of 10 ml $Na_2S_2O_3$ (sat.). The phases were separated and organic phase was washed with $NaHCO_3$ (sat.). The water phases were extracted with 3 times with 20 ml dry CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4, filtered and evaporated. Flash chromatography (SiO_2, heptane/Et_2O 85:15) afforded the required isomer **13a** as a colourless oil in 79% (0.45 g) yield. ^{1}H NMR (400 MHz, $CDCl_3$) 7.29 (1 H, d, 2.5), 4.41 (1 H, s), 3.84 (2 H, m), 3.72 (3 H, s), 2.70 (1 H, dddd, 16.5, 8.3, 6.1, 2.5), 2.05 (1 H, dd, 13.9, 7.2), 1.97 (1 dddd, 16.5, 13.2, 6.8, 3.3), 1.89 (1 H, m), 1.83 (1 H, ddd, 13.2, 7.2, 5.3), 1.66 (1 H, m), 1.62 (1 H, s), 1.58 (1 H, m), 1.30 (1 H, td, 13.2, 8.3), 1.12 (1 H, m), 1.08 - 1.04 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 165.4, 153.9, 126.3, 63.6, 63.3, 60.7, 51.5, 39.7, 38.0, 35.8, 30.1, 27.5, 23.2, 18.0, 11.9. HRMS calcd for $C_{22}H_{38}O_4NaSi$ [M + Na]: 417.2437, found: 417.2413.

rac-(1a*S*,2*S*,3a*S*,5*R*,7a*S*)-Methyl 2-phenyl-5-(2-((triisopropylsilyl)oxy)ethyl)-1a,2,3,3a,4,5-hexahydroindeno [1,7a-*b*]oxirene-7-carboxylate(**13b**): **13b** was prepared in the same way as **13a**, starting from **12b** (2.64 g, 5.8 mmol). The crude product (2.71 g) was purified by flash chromatography (SiO_2, heptane/Et_2O 85:15). **13b** was obtained as a colourless oil (0.43 g, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.42 (2 H, dd, 8.2, 1.3), 7.34 (1 H, d, 0.9), 7.31 (2 H, td, 8.2, 7.3), 7.24 (1 H, td, 7.3, 1.3), 4.57 (1 H, s), 3.85 (2 H, m), 3.72 (3 H, s), 3.16 (1 H, dd, 12.1, 7.2), 2.75 (1 H, m), 2.12 (1 H, m), 2.04 (1 H, m), 1.92 (1 H, dt, 12.1, 7.2), 1.86 (1 H, dt, 15.2, 6.8), 1.67 (1 H, ddd, 15.2, 10.8, 6.8), 1.42 (1 H, m), 1.32 (1 H, dd, 12.1, 11.5), 1.17 - 1.03 (21 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) 165.4, 154.1, 141.6, 128.4, 127.7, 126.6, 126.1, 66.9, 62.3, 60.6, 51.6, 45.9, 40.2, 38.0, 35.6, 32.3, 30.0, 18.0, 12.0. HRMS calcd for $C_{28}H_{42}O_4SiNa$ [M + Na]: 493.2750, found: 493.2773.

rac-(2a*S*,4*R*,5a*R*,7a*R*)-2a-Hydroxy-4-(2-hydroxyethyl)-4,5,5a,6,7,7a-hexahydroindeno [1,7-*bc*]furan-2(2a*H*)-one(**3**): **13a (**0.45 g, 1.2 mmol) was dissolved in 5 ml THF and a solution of $LiOH{\cdot}H_2O$ (0.12 g, 2.8 mmol) in 5 ml H_2O was added at room temperature. The reaction mixture was stirred for 3 days until a TLC analysis revealed that the starting material was consumed. After the addition of 5 ml THF and 5 ml of H_2SO_4 (10%), the mixture was stirred at room temperaturefor 3 days. The reaction mixture was quenched by the addition of 3 ml $NaHCO_3$ (sat.) and extracted 3 times with 20 ml EtOAc, the combined organic phases were dried with Na_2SO_4 and concentrated by evaporation. The crude product was purified by chromatography (SiO_2, $CHCl_3$/MeOH 9:1) to afford 0.13 g (50%) of **3** as a colourless oil. ^{1}H NMR (500 MHz, CD_3OD) 7.11 (1 H, d, 3.1), 4.69 (1 H, dd, 7.5, 2.7), 3.71 (2 H, t, 6.4), 2.70 (1 H, m), 2.43 (1 H, dtd, 10.5, 7.9, 5.0), 2.27 (1 H, m), 2.10 (1 H, ddt, 14.3, 10.5, 7.5), 1.87 (1 H, m), 1.76 (1 H, td, 13.2, 6.4), 1.69 (1 H, dddd, 14.3, 7.5, 5.0, 2.7), 1.65 (1 H, m), 1.18 (1 H, m), 1.10 (1 H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 172.2, 149.7, 132.9, 91.9, 83.0, 60.9, 44.4, 39.1, 32.7, 32.6, 32.5, 32.4. HRMS calcd for $C_{12}H_{16}O_4Na$ [M + Na]: 247.0946, found: 247.0932.

rac-(2a*S*,4*R*,5a*S*,7*S*,7a*R*)-2a-Hydroxy-4-(2-hydroxyethyl)-7-phenyl-4,5,5a,6,7,7a-hexahydroindeno [1,7-*bc*] furan-2(2a*H*)-one(**4**): **4** was prepared in the same way as **3**, starting from **13b** (0.13 g, 0.4 mmol). The crude product was purified by chromatography (SiO_2,$CHCl_3$/MeOH 9:1) to afford **3** (60 mg, 73%) as a colourless oil. ^{1}H (500 MHz, $CDCl_3$) 7.24 (2 H, t, 7.4), 7.17 (1 H, td, 7.4, 2.3), 7.12 (1 H, d, 3.4), 7.09 (2 H, dt, 7.4, 2.3), 4.82 (1 H, dd, 6.3, 1.0), 3.72 (2 H, m), 3.47 (1 H, dt, 13.2, 6.3), 2.78 (1 H, m), 2.56 (1 H, dddd, 12.0, 8.1, 6.4, 3.8), 2.32 (1 H, ddd, 13.5, 8.1, 7.8), 2.06 (1 H, dddd, 13.2, 6.4, 6.3, 1.0), 1.72 (2 H, m), 1.36 (1 H, td, 13.2, 12.0), 1.04 (1 H, ddd, 13.5, 12.0, 3.8). ^{13}C NMR (125 MHz, $CDCl_3$) 170.3, 148.4, 136.8, 131.4, 128.3, 128.1, 126.7, 90.9, 81.2, 59.8, 48.2, 42.2, 37.7, 37.4, 31.2, 30.1. HRMS calcd for $C_{18}H_{20}O_4Na$ [M + Na]: 323.1259, found: 323.1283.

2-(-2a-Hydroxy-2-oxo-2,2a,4,5,5a,6,7,7a-octahydroindeno [1,7-*bc*]furan-4-yl)ethyl 6-(5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1*H*-thieno [3,4-*d*]imidazol-4-yl)pentanamido)hexanoate(**5**): To a dried flask containing the *N*-(+)-

biotinyl-6-aminohexanoic acid (50 mg, 0.13 mmol) was added thionyl chloride (0.50 ml, 6.3 mmol) under a nitrogen atmosphere and the mixture was stirred for 40 min at room temperature. The excess thionyl chloride was subsequently removed under reduced pressure and the crude product was used directly in the next step. **3** (19.2 mg, 0.09 mmol) was dissolved in 1.5 ml of freshly distilled MeCN and the previously prepared acid chloride dissolved in 1.5 ml MeCN was added dropwise under a nitrogen atmosphere**.** The reaction mixture was stirred for 5 h at room temperature and then concentrated under reduced pressure. The crude product was purified by chromatography (SiO_2, $CHCl_3$/MeOH 97:3) to afford **5** (9 mg, 19%). The two diastereomers have identical NMR data. ^{1}H (500 MHz, CD_3OD) 7.11 (1 H, d, 3.2), 4.70 (1 H, dd, 7.3, 2.4), 4.52 (1 H, dd, 7.8, 4.5), 4.33 (1 H, dd, 7.8, 4.5), 4.24 (2 H, m), 3.23 (1 H, m), 3.17 (2 H, t, 6.9), 2.94 (1 H, dd, 12.8, 4.5), 2.72 (1 H, d, 12.8), 2.66 (1 H, tdd, 12.2, 7.1, 3.2), 2.44 (1 H, dtd, 10.5, 7.5, 2.4), 2.35 (2 H, t, 7.8), 2.29 (1 H, m), 2.20 (2 H, t, 7.4), 2.11 (1 H, ddt, 14.5, 10.5, 7.3), 1.89 (1 H, td, 14.5, 7.5), 1.81 (1 H, m), 1.73 (1 H, m), 1.77 - 1.55 (7 H, m), 1.52 (2 H, dt, 12.2, 6.9), 1.44 (2 H, m), 1.35 (2 H, m), 1.16 (1 H, m), 1.09 (1 H, m). ^{13}C NMR (125 MHz, CD_3OD) δ 176.2, 175.4, 172.0, 166.2, 148.9, 133.4, 91.9, 83.0, 63.7, 63.6, 62.0, 57.1, 44.5, 41.1, 40.4, 36.9, 35.1, 35.0, 34.8, 32.9, 32.9, 32.7, 32.5, 30.2, 29.9, 29.6, 27.6, 27.1. HRMS calcd for $C_{28}H_{42}N_3O_7S$ [M + H]: 564.2743, found: 564.2744.

2-(-2a-Hydroxy-2-oxo-7-phenyl-2,2a,4,5,5a,6,7,7a-octahydroindeno [1,7-*bc*]furan-4-yl)ethyl 6-(5-((3a*S*,4*S*, 6a*R*)-2-oxohexahydro-1*H*-thieno [3,4-*d*]imidazol-4-yl)pentanamido)hexanoate(**6**): **6** was prepared and purified in the same way as **5**, starting from **4** (10 mg, 0.033 mmol) to afford **6** (9.7 mg, 45%) as a colourless oil. The two diastereomers have identical NMR data. ^{1}H (500 MHz, CD_3OD) 7.27 (2 H, m), 7.20 (1 H, m), 7.17 (2 H, dd, 6.1, 2.7), 7.15 (1 H, s), 4.86 (1 H, d, 6.5), 4.47 (1 H, dd, 7.9, 4.8), 4.28 (1 H, m), 4.28 (2 H, dd, 13.1, 5.3), 3.58 (1 H, dt, 13.5, 6.5), 3.21 (1 H, m), 3.16 (2 H, dd, 8.9, 7.4), 2.92 (1 H, dt, 12.8, 4.8), 2.79 (1 H, m), 2.70 (1 H, dd, 12.8, 3.8), 2.62 (1 H, dddd, 10.0, 8.1, 6.5, 3.6), 2.36 (3 H, m), 2.19 (2 H, td, 7.4, 2.1), 2.09 (1 H, ddd, 13.5, 6.5, 5.5), 1.95 (1 H, td, 13.1, 6.2), 1.86 (1 H, m), 1.73 (1 H, m), 1.69 - 1.55 (6 H, m), 1.51 (2 H, m), 1.44 (2 H, m), 1.36 (2 H, m), 1.20 (1 H, ddd, 13.2, 8.1, 3.9). ^{13}C NMR (125 MHz, CD_3OD) 176.1, 175.4, 172.0, 166.3, 148.9, 138.9, 133.2, 129.7, 129.3, 127.8, 92.6, 82.7, 63.7, 63.5, 61.7, 57.2, 49.6, 43.6, 41.2, 40.3, 38.7, 37.0, 35.2, 35.0, 33.1, 30.82, 30.2, 29.9, 29.6, 27.6, 27.1, 25.9. HRMS calcd for $C_{34}H_{46}N_3O_7S$ [M + H]: 640.3056, found: 640.3051.

(*S*)-*N*-Methoxy-*N*-methyl-2-phenylpent-4-enamide(**17**):To a solution of (*S*)-2-phenylpent-4-enoic acid (**16**) (6.8 g, 38 mmol), prepared from phenylacetic acid (**14**) according to reference 16 and identical in all aspects with the reported compound, in dry CH_2Cl_2 (4 ml) was added freshly distilled $SOCl_2$ (11.4 g, 96 mmol) at 0˚C. The resulting brown mixture was stirred overnight at room temperature, where after the volatiles were removed under reduced pressure. The acid chloride, obtained as a black solid (7.8 g), was then dissolved in dry CH_2Cl_2 (40 ml) and cooled to 0˚C before the addition of *N*-methyl-*O*-methylhydroxylamine hydrochloride (4.3 g, 44 mmol) under N_2. After stirring at 0˚C for 2 h, pyridine (6.7 g, 84 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed under vacuum, and the remaining pale yellow solid was dissolved in a 1:1mixture of Et_2O/CH_2Cl_2 (50 mL) and washed with brine (50 ml).The organic layer was dried over $MgSO_4$, and the solvents were removed under vacuum. The crude product was purified using flash chromatography (SiO_2, heptane/EtOAc 8:2) to afford 3.06 g (37% over two steps from **14** via **16**) of **17** as a colourless oil. $[\alpha]_D^{20}$ +75.0 (*c* 0.2 in $CDCl_3$). ^{1}H (400 MHz, $CDCl_3$) 7.37 - 7.21 (5 H, m), 5.75 (1 H, ddt, J 17.0, 10.2, 6.9), 5.06 (1 H, ddd, J 17.0, 3.3, 1.5), 4.99 (1 H, ddd, J 10.2, 3.3, 1.0), 4.09 (1 H, m), 3.47 (3 H, s), 3.17 (3 H, s), 2.85 (1 H, m), 2.47 (1 H, m). ^{13}C (100 MHz, $CDCl_3$) 177.60, 139.64, 136.12, 128.56, 128.17, 126.99, 116.56, 61.29, 51.20, 38.21, 32.26. HRMS calcd for $C_{13}H_{18}NO_2$ [M + H]: 220.1338, found: 220.1343

(*S*)-4-Phenylhept-6-en-1-yn-3-one(**18**):To a solution of **17** (3.1 g, 14 mmol) in 20 ml of dry THF at –78˚C was added dropwise a solution of ethynylmagnesium bromide (110 ml, 0.5 M in THF, 55 mmol) under N_2. The mixture was stirred at −78˚C for 30 min and then allowed to warm to room temperature overnight. The reaction was quenched with 70 ml of NH_4Cl (sat). The layers were separated and the aqueous phase was extracted 3 times with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (SiO_2, heptane/EtOAc 7:3) afford 2.6 g (quantitative yield) of the corresponding ynone **18**. $[\alpha]_D^{20}$ +73.3 (*c* 0.3 in $CDCl_3$). ^{1}H (400 MHz, $CDCl_3$) 7.37 (2 H, td, J 8.0, 1.9), 7.32 (1 H, tt, J 8.0, 1.9), 7.27 (2 H, dd, J 8.0, 1.9), 5.70 (1 H, ddt, J 17.0, 10.2, 7.1), 5.08 (1 H, ddd, J 17.0, 3.1, 1.5), 5.02 (1 H, ddd, J 10.2, 3.1, 1.1), 3.86 (1 H, t, J 7.1), 3.19 (1 H, s), 2.93 (1 H, dtd, J 14.4, 7.1, 1.1), 2.56 (1 H, dtd, J 14.4, 7.1, 1.5). ^{13}C (100 MHz, $CDCl_3$) 184.61, 134.74, 128.90, 128.69, 127.81, 117.33, 80.17, 77.20, 60.39, 35.38.HRMS calcd for $C_{13}H_{13}O$ [M + H]: 185.0961, found: 185.0960.

(*S*)-4-Phenylhept-6-en-1-yn-3-ol(**19**):**18** (3.4 g, 18 mmol) was dissolved in dry THF (100 ml) and cooled to

−78˚C before the addition of DIBAL (46 ml, 1M in THF) under N_2. The reaction mixture was stirred for 3 h at –78˚C and then quenched by the addition of 3 ml of MeOH and 20 ml of a solution of Na/K tartrate (sat), extracted with EtOAc (40 ml x 3), dried over Na_2SO_4 and concentrated under reduced pressure. **19** (3.2 g, 92%) was obtained as a 1:0.24 diasteromeric mixture and used in the next step without further purification. *Diastereomer* a). ^{1}H (400 MHz, $CDCl_3$) 7.39 - 7.28 (5 H, m), 5.69 (1 H, ddd, 17.1, 10.1, 5.8), 5.07 (1 H, ddd, J 17.1, 3.3, 1.5), 4.98 (1 H, ddt, J 10.1, 3.3, 1.1), 4.55 (1 H, dd, J 5.8, 2.2), 2.98 (1 H, dt, J 9.2, 5.8), 2.78 (1 H, ddd, J 14.4, 5.8, 1.5), 2.57 (1 H, dd, 14.4, 9.2, 1.1), 2.53 (1 H, d, J 2.2). ^{13}C (100 MHz, $CDCl_3$) 139.17, 135.99, 128.86, 128.41, 127.22, 116.97, 83.25, 74.73, 65.72, 51.66, 35.35. *Diastereomer* b). ^{1}H (400 MHz, $CDCl_3$) 7.39 - 7.28 (5 H, m), 5.69 (1 H, ddd, 17.1, 10.1, 5.8), 5.09 (1 H, ddd, J 17.1, 3.3, 1.5), 4.98 (1 H, ddt, J 10.1, 3.3, 1.1), 4.59 (1 H, dd, J 5.8, 2.2), 3.03 (1 H, dt, J 9.2, 5.8), 2.78 (1 H, ddd, J 14.4, 5.8, 1.5), 2.57 (1 H, dd, 14.4, 9.2, 1.1), 2.49 (1 H, d, J 2.2). ^{13}C (100 MHz, $CDCl_3$) 139.02, 135.84, 129.19, 128.27, 128.00, 116.92, 83.25, 75.05, 65.58, 50.90, 35.29. HRMS calcd for $C_{13}H_{14}ONa$ [M + Na]: 209.0942, found: 209.0946.

Methyl ((4*S*,*Z*)-4-phenyldeca-1,8-dien-6-yn-5-yl) carbonate(**20**): The Sonogashira reaction was performed in the corresponding way as when **8a**/**8b** was transformed to **10a**/**10b**, starting from **19** (3.4 g, 18 mmol) and *cis*-1-bromo-prop-1-ene (6.6 g, 55 mmol), and the crude product was used directly for the preparation of the dienyne carbonate **20** as described for **11a**/**11b**. **20** (0.70 g, 13%) was obtained as a 1:0.15 diastereomeric mixture after purification by flash chromatography (Si_2O, heptane/CH_2Cl_2 1:1). *Diasteromer a*. ^{1}H (400 MHz, $CDCl_3$) 7.36 - 7.22 (5 H, m), 6.03 (1 H, dq, J 10.7, 6.9), 5.66 (1 H, ddt, J 17.2, 10.2, 7.0), 5.59 (1 H, dd, J 6.0, 1.9), 5.50 (1 H, ddd, 10.7, 1.7, 1.5), 5.03 (1 H, ddd, J 17.2, 3.4, 1.4), 4.96 (1 H, ddt, J 10.2, 3.4, 1.1), 3.76 (3 H, s), 3.14 (1 H, dt, J 6.0, 5.1), 2.77 (1 H, m), 2.65 (1 H, m), 1.82 (3 H, dd, J 6.9, 1.7). δ C (100 MHz, $CDCl_3$) 154.87, 140.19, 138.92, 135.46, 128.89, 128.25, 127.15, 116.99, 109.12, 88.69, 84.65, 71.89, 54.89, 49.70, 35.08, 16.05. *Diasteromer b.* ^{1}H (400 MHz, $CDCl_3$) 7.36 - 7.22 (5 H, m), 6.00 (1 H, dq, J 10.7, 6.9), 5.66 (1 H, ddt, J 17.2, 10.2, 7.0), 5.59 (1 H, dd, J 6.0, 1.9), 5.45 (1 H, ddd, 10.7, 1.7, 1.5), 5.01 (1 H, ddd, J 17.2, 3.4, 1.4), 4.96 (1 H, ddt, J 10.2, 3.4, 1.1), 3.80 (3 H, s), 3.14 (1 H, dt, J 6.0, 5.1), 2.77 (1 H, m), 2.65 (1 H, m), 1.74 (3 H, dd, J 6.9, 1.7). ^{13}C (100 MHz, $CDCl_3$) 154.87, 139.97, 139.17, 135.46, 128.78, 128.19, 127.11, 116.96, 109.09, 88.69, 84.65, 71.89, 54.89, 49.41, 35.47, 15.95. HRMS calcd for $C_{18}H_{20}O_3Na$ [M + Na]: 307.1310, found: 307.1331

Methyl 6-methyl-(2*R*)-phenyl-2,6,7,7a-tetrahydro-1*H*-indene-4-carboxylate(**21**): The diene **21** was obtained following the same procedure as when **12a**/**12b** was prepared from **11a**/**11b**, starting with **20** (0.70 g, 2.4 mmol). **21** (0.32 g, 48%) was obtained as a 1:0.7 mixture of two diasteromers as a yellow oil, after flash chromatography (SiO_2, heptane/Et_2O 95:5). *Diasteromer* a).^{1}H (400 MHz, $CDCl_3$) 7.35 - 7.17 (5 H, m), 6.81 (1 H, t, J 2.8), 6.33 (1 H, t, J 2.7), 4.04 (1 H, dd, J 3.0, 1.6), 3.79 (3H, s), 2.98 (1 H, m), 2.65 (1 H, m), 2.12 (1 H, m), 2.06 (1 H, t, J 5.4), 1.99 (1 H, dt, J 12.7, 9.6), 1.14 (3 H, dd, J 7.3, 5.7), 1.07 (1 H, m). ^{13}C (100 MHz, $CDCl_3$) 166.93, 166.91, 147.73, 145.90, 138.18, 130.52, 128.34, 127.19, 125.96, 51.59, 50.32, 43.34, 39.69, 38.45, 32.92, 20.74. *Diasteromer* b). ^{1}H (400 MHz, $CDCl_3$) 7.35 - 7.17 (5 H, m), 6.81 (1 H, t, J 2.8), 6.41 (1 H, t, J 2.8), 4.00 (1 H, ddt, J 10.5, 3.0, 1.6), 3.80 (3 H, s), 2.86 (1 H, ddt, J 13.0, 9.5, 2.8), 2.65 (1 H, m), 2.59 (1 H, dt, J 12.2, 7.1), 2.06 (1 H, t, J 5.4), 1.37 (1 H, dt, J 12.3, 10.3), 1.16 (3 H, dd, J 7.3, 5.7), 1.07 (1 H, m). ^{13}C (100 MHz, $CDCl_3$) 166.93, 166.91, 147.60, 145.50, 138.00, 129.53, 128.36, 127.43, 126.11, 51.59, 51.27,44.66, 42.09, 38.34, 33.14, 20.83. HRMS calcd for $C_{18}H_{21}O_2$ [M + H]: 269.1542, found: 269.1550.

(1a*S*,2*S*,3a*S*,5*S*,7a*S*)-Methyl 5-methyl-2-phenyl-1a,2,3,3a,4,5-hexahydroindeno [1,7a-*b*]oxirene-7-carboxylate (**22**)**:22** was prepared following the same procedure as when **13a**/**13b** was prepared from **12a**/**12b**, starting from **21** (0.31 g, 1.17 mmol). **22** (0.14 g, 42%) was obtained after flash chromatography (SiO_2, heptane/Et_2O 85:15). $[\alpha]_D^{20}$ + 8.5 (*c* 4.1 in $CDCl_3$). ^{1}H (400 MHz, $CDCl_3$) 7.42 (1 H, m), 7.32 (2 H, m), 7.23 (2 H, m), 7.17 (1 H, dd, J 7.4, 2.2), 4.57 (1 H, s), 3.75 (3 H, s), 3.52 (1 H, m), 2.56 (1 H, m), 2.14 (1 H, m), 1.94 (1 H, m), 1.90 (1 H, m), 1.64 (1 H, m), 1.40 (1 H, ddd, J 14.8, 9.7, 7.5), 1.20 (3 H, dd, J 7.3). ^{13}C (100 MHz, $CDCl_3$) 165.40, 155.17, 141.93, 128.61, 127.49, 126.58, 125.84, 66.40, 64.14, 62.15, 51.71, 44.67, 37.81, 33.76, 32.19, 20.40. HRMS calcd for $C_{18}H_{21}O_3$ [M + H]: 285.1491, found: 285.1472.

(2a*S*,4*S*,5a*S*,7*S*,7a*R*)-2a-Hydroxy-4-methyl-7-phenyl-4,5,5a,6,7,7a-hexahydroindeno [1,7-*bc*]furan-2(2a*H*)-one(**2**):**2** was prepared in the same way as when **13a**/**13b** was transformed to **3**/**4**, starting from **22** (0.14 g, 0.5 mmol). The crude product was purified by chromatography (SiO_2, heptane/EtOAc 60:40.) to afford **2** (30 mg, 22%) as a colourless oil. $[\alpha]_D^{20}$ +59 (*c* 12.5 in $CDCl_3$). ^{1}H (400 MHz, $CDCl_3$) 7.30 (2 H, m), 7.23 (1 H, m), 7.13 (2 H, m), 7.10 (1 H, d, J 3.2), 4.90 (1 H, dd, J 6.7, 1.4), 3.52 (1 H, dt, J 13.4, 6.7), 2.77 (1 H, m), 2.62 (1 H, m), 2.36 (1 H, dt, J 14.0, 7.6), 2.12 (1 H, dtd, J 12.7, 6.7, 1.4), 1.45 (1 H, dd, J 13.4, 12.7), 1.25 (3 H, d, J 7.3), 1.12 (1 H, m). ^{13}C (100 MHz, $CDCl_3$) 170.0, 150.3, 136.8, 130.7, 128.4, 128.2, 126.8, 90.5, 81.3, 48.2, 42.2, 37.6, 31.6, 29.1,

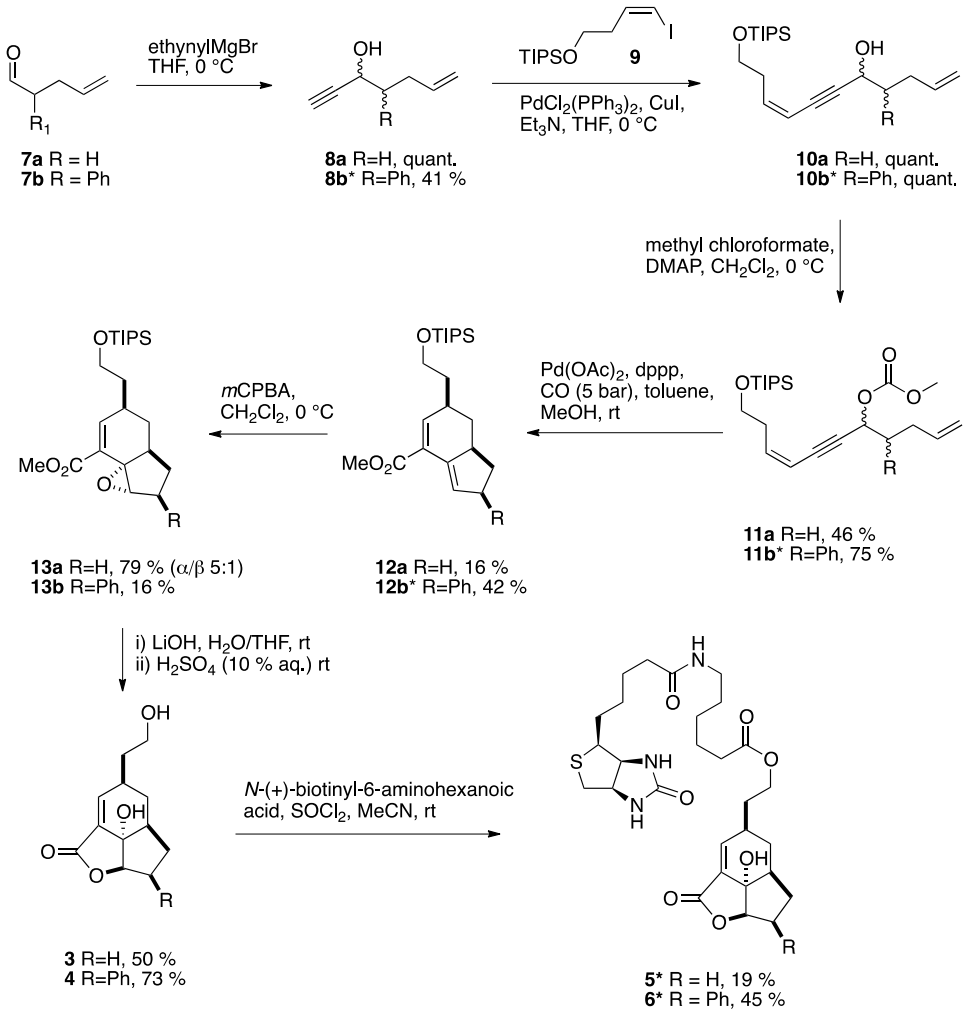
20.6. HRMS calcd for $C_{17}H_{19}O_3$ [M + H]: 271.1334, found: 271.1351

WST-1 cell proliferation assay

The functional activity of **1**, **2**, **3** and **4** were evaluated using a WST-1 proliferation assay with DU145 cells (ATCC, American Type Culture Collection, LGC Standards AB, Borås, Sweden) which expresses constitutively activated STAT3, as previously described [6]. In short, DU145 cells were cultured in 96-well plates (2000 cells/well in 200 µl of medium) and allowed to set for 24 h. The cells were treated with the test items for 72 h. Samples were made in triplicate. 20 µl WST-1 solution (Roche Applied Science) was added per well and incubated at 37˚C for 4 h. The absorbance of each well was measured using a scanning multi-well spectrophotometer, ELISA reader at a wavelength of 450 nm and reference wavelength of 690 nm. The results are presented as per cent of untreated control cells.

3. Results and Discussion

Our aim was to introduce a suitable functional group on the C-4 methyl group, as a handle to attach biotin via a linker. Initial attempts to introduce an alkyne or an azide to introduce the biotin group by "click chemistry" [12] failed. Instead a primary hydroxyl group was chosen as the functional group handle to attach biotin through an ester coupled linker, and the synthetic procedures for obtaining the galiellalactone analogues **3** and **4** as well as their biotinylated derivatives **5** and **6** are summarized in **Scheme 1**.



Scheme 1. Synthesis of 8-hydroxymethyl galiellalactone biotin 6-aminohexanoic acid amide ester (**5**) and 8-hydroxymethyl-7-phenyl galiellalactone biotin 6-aminohexanoic acid amide ester (**6**). *Intermediates **8b**, **10b**, **11b** and **12b** as well as the end products **5** and **6** were obtained as mixtures of diastereomers.

Ethynyl magnesium bromide was added to 5-pentenal (**7a**) to give the propargylic alcohol **8a** in a quantative yield. A Sonogashira coupling of the terminal alkyne of **8a** with the known *cis*-iodoalkene **9** [13] containing a TIPS-protected hydroxyl group gave the alkenyne **10a**. Thiswas treated with methyl chloroformate in the presence of DMAP to give the carbonate **11a**, the key substrate for the tandem palladium catalyzed carbonylation/intramolecular Diels-Alder reaction. The ring forming transformation using $Pd(OAc)_2$ and 1,3-bis(diphenylphosphino)propane under a CO atmosphere [8] gave the bicylic product **12a** with the TIPS protected hydroxyl group intact. Regioselective epoxidation of the electron-rich double bond with *m*-CPBA gave the desired epoxide as a 5:1 *α*:*β* diastereomeric mixture, from which the desired isomer **13a** could be isolated. **13a** was subjected to the conditions bringing about the lactone formation [8], in this step the TIPS protecting group was lost and 8-hydroxymethyl galiellalactone **3** was isolated in 50% yield. The synthesis of **3** proceeded with good or acceptable yields, except for the tandem palladium catalyzed carbonylation/intramolecular Diels-Alder reaction (from **11a** to **12a**), but this transformation gives poorer yields when the cyclopentene ring is unsubstituted. [8]

N-(+)-biotinyl-6-aminohexanoic acid had previously been described as a suitable reagent for making biotin conjugates of a biologically active compound [14]. However, attempts to couple *N*-(+)-biotinyl-6-aminohexanoic acid using standard reagents for esterification failed, presumably because a base is used and the tricyclic galiellalactone system is sensitive to basic conditions. Galiellalactone (**1**) is relatively stable in acidic conditions and towards acid chlorides, so by first treating *N*-(+)-biotinyl-6-aminohexanoic acid with thionyl chloride to generate the corresponding acid chloride and then adding the alcohol **3** in acetonitrile the C-8 biotin conjugate **5** was prepared and could be isolated in 19% yield.

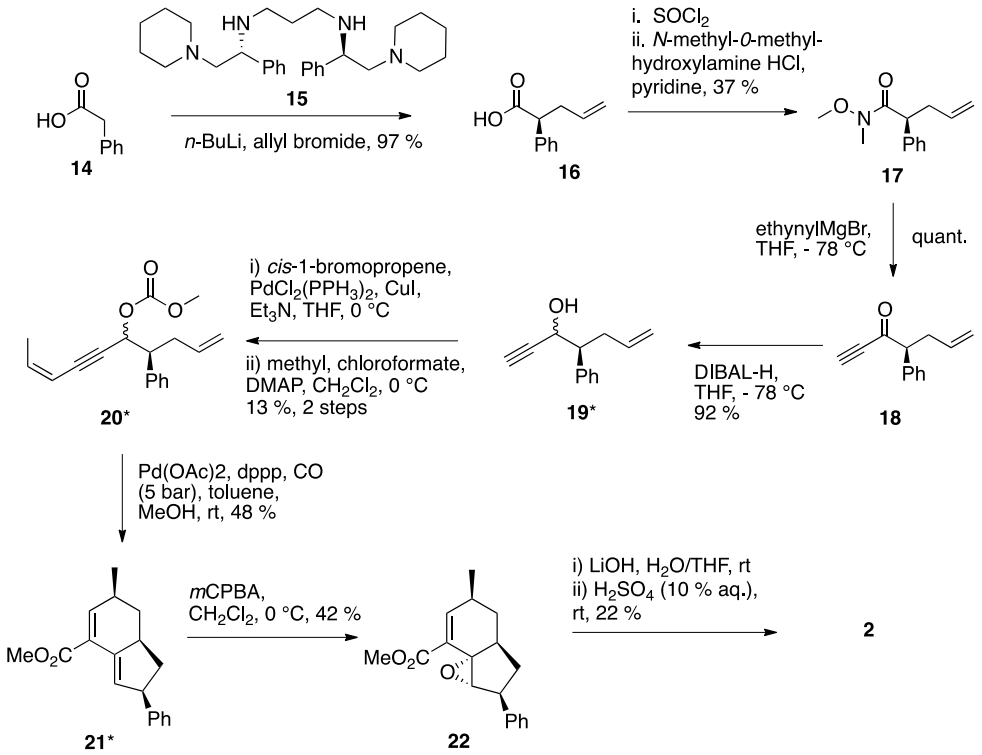
The corresponding biotin labeled 7-phenyl substituted derivative **6** was synthesized in an analogous manner. Starting from aldehyde **7b**, prepared by a DIBAL-H reduction of the corresponding 2-phenyl pent-4-ene acid ester, the key intermediate **11b** could be obtained in three steps as a mixture of diastereomers. As we previously have observed, the tandem carbonylation/intramolecular Diels-Alder reaction gives a higher yield when a substituent in position 7 is present [8], and the cyclization of **11b** proceeds in a 42% yield although the product is obtained as a mixture of two diastereomers. The epoxide **13b** was isolated as a pure diastereomer but in a relatively poor yield, due to reduced stereoselectivity in the epoxidation, but the lactone formation to afford **4** runs smoother. The relative configuration of **3** as well as **4** was established by NMR NOESY experiments. Finally, the synthesis of **6** was accomplished by the esterification with *N*-(+)-biotinyl-6-aminohexanoic acid chloride.

To investigate the effect of a phenyl group in position 7, an asymmetric synthesis of 7-phenyl galiellalactone (**2**) was devised. The key objective was to obtain the substituted propargylic alcohol **19** with the absolute stereochemistry of C-4 set as shown in **Scheme 2**, as this would influence the stereochemistry of the subsequent stereocenters generated in the cyclization step. By applying a procedure developed for the enantioselective direct alkylation of arylacetic acids [15] using Koga's base (**15**) [16], (2*S*)-phenylpent-4-enoic acid (**16**) [17] was prepared from phenylacetic acid (**14**). By this procedure, **16** is obtained with 88% ee [17], and the material obtained here was identical to that reported. The acid **16** was converted to the corresponding Weinreb amide **17**, which subsequently was reacted with ethynyl magnesium bromide to give the ynone **18**. Reduction of the ketone with DIBAL gave the desired propargylic alcohol **19**. The final steps from **19** via **20**, **21**, and **22** to **2** followed the procedure outlined in **Scheme 2**. **19** and **20** were obtained as mixtures of two diastereomers, in the ratios 1:0.24 and 1:0.15, but both diastereomers of **20** give the same product in the cyclization step. **21** was obtained as a mixture of two diastereomers in the ratio 1:0.7, the major is shown in **Scheme 2** and this was readily epoxidized to **22**.

To determine how the structural changes in **2**, **3** and **4** affected their ability to block STAT3 signaling compared to galiellalactone (**1**), they were assayed for their ability to inhibit proliferation of DU145 prostate cancer cells which express active STAT3 *in vitro*, using a WST-1 assay (see **Figure 2**) [6]. The hydroxyl methyl analogue **3** had significantly reduced ability to inhibit proliferation (GIC_{50} = 32 µM) compared to **1** (GIC_{50} = 3.7 µM), but the biological activity was restored when a phenyl group was added in position 7 as observed with analogue **4** (GIC_{50} = 4.6 µM). Compared with the natural product, the 7-phenyl analogue **2** has a slightly improved potency (GIC_{50} = 2.9 µM).

4. Conclusion

The biotinylated analogues **5** and **6** will be used in various biological studies to elucidate the mechanism of action of galiellalactone, starting with its effect on STAT3 signaling [5]. The fact that both **5** and **6** retain the



Scheme 2. Asymmetric synthesis of 7-phenyl galiellalactone (**2**). *Intermediates **19**, **20** and **21** were obtained as mixtures of diastereomers.

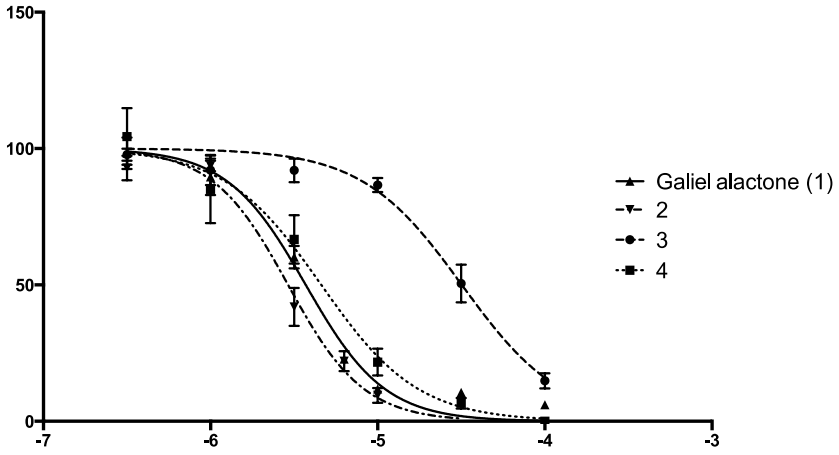


Figure 2. Cell proliferation dose response curve. Galiellalactone (**1**), **2**, **3** and **4** inhibit proliferation of DU145 cells with GIC_{50} values of 3.7 µM, 2.9 µM, 32 µM and 4.6 µM, respectively after 72 h. As discussed below, the biotinylated derivatives **5** and **6** retained the inhibitory effect.

ability to inhibit the proliferation of DU145 cells expressing constitutively active STAT3 (with the GIC_{50} values 6.6 and 14 µM, respectively, in the same WST-1 assay [5]) demonstrates that the attachment of biotin via a linker to position 4 of **1** produces cell permeable covalent STAT3 inhibitors that can be used as important tools for elucidating the mechanism of action of **1**. With the biotinylated analogues synthesized in this work, in combination with biochemical methods, protein isolation and mass spectrometry, it was possible to show that **1** is a di-

rect inhibitor of STAT3 by alkylating critical cysteine residues [6].

Acknowledgements

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