Enantiospecific synthesis of the heparanase inhibitor (+)-trachyspic acid and stereoisomers from a common precursor†

Steven C. Zammit,[a] Vito Ferro,[b] Edward Hammond[b] and Mark A. Rizzacasa*[a]

Received 6th June 2007, Accepted 20th June 2007
First published as an Advance Article on the web 26th July 2007
DOI: 10.1039/b708594j

The total synthesis of natural (+)-trachyspic acid and its enantiomer is described starting from a common 2-deoxy-D-ribose derivative. The synthesis of the corresponding C3 epimers from the same starting material is also described. Each stereoisomer was assayed for heparanase inhibition.

Introduction

Heparanase is an endo-β-glucuronidase that cleaves the heparan sulfate (HS) side chains of proteoglycans that are found on cell surfaces and as a major constituent of the extracellular matrix (ECM) and basement membranes surrounding cells.[1] The degradation of the ECM by heparanase facilitates the spread of metastatic tumor cells and leukocytes by allowing them to pass into the blood stream and lodge in remote sites, where they can form secondary tumors or cause inflammation, respectively. In addition, heparanase is able to liberate HS-bound angiogenic growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF-1 and FGF-2) which promote tumor growth and angiogenesis. Because of its pivotal role in these processes, heparanase is an attractive target for the development of antitumor, antimetastasis or anti-inflammatory drugs.[2]

Trachyspic acid (**1**, Fig. 1) was isolated from the culture broth of *Talaromyces trachyspermu* SANK 12191 and was identified as a potent inhibitor of heparanase with an IC_{50} of 36 μM.[3]

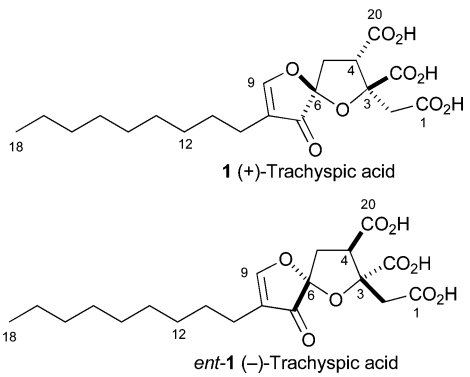
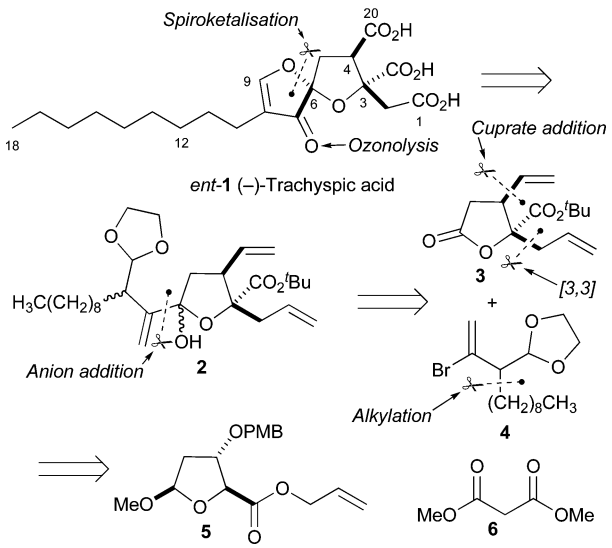


Fig. 1 Structures of (+)-(3*S*,4*S*,6*S*)-trachyspic acid (**1**) and of (−)-(3*R*,4*R*,6*R*)-trachyspic acid (*ent*-**1**).

Its structure was determined by NMR analysis and degradation experiments and the relative configuration was confirmed by a total synthesis of racemic **1**.[4] Compound **1** is a member of a family of 2-alkylcitrate type natural products which includes cinatrin B[5] and citrafungin A.[6] We have recently reported the total synthesis of (−)-(3*R*,4*R*,6*R*)-trachyspic acid (*ent*-**1**) and confirmed the absolute configuration of natural (+)-(3*S*,4*S*,6*S*)-trachyspic acid (**1**).[7] In this article we report the full details of this work as well as the synthesis of natural (+)-trachyspic acid (**1**) and the corresponding C3 epimers from a common chiral pool precursor. Each isomer was then tested for inhibition of heparanase.

Results and discussion

Our initial retrosynthetic analysis of (−)-trachyspic acid (*ent*-**1**) is shown in Scheme 1. At the onset of this work, the relative and absolute configuration of **1** was unknown. We selected *ent*-**1** as our initial target and envisaged that it could be synthesised from the lactol precursor **2** by acid hydrolysis of the dioxolane and spirocyclisation of the resultant aldehyde followed by lactol acetylation[4] and global ozonolysis of three terminal alkenes.

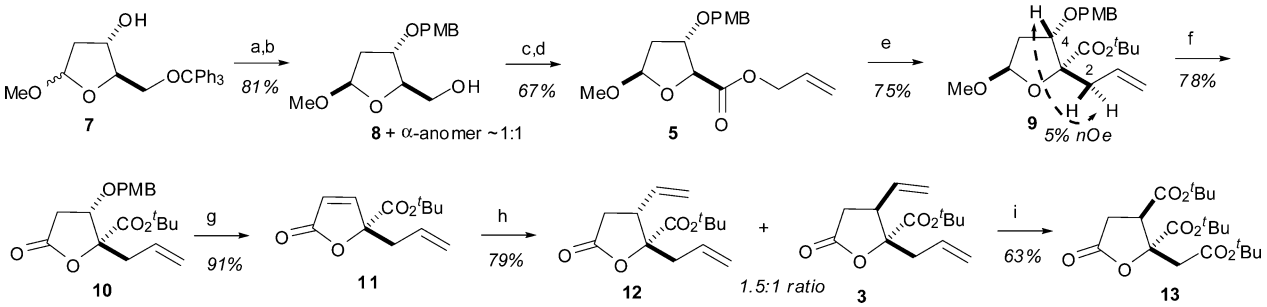


Scheme 1 Initial retrosynthetic analysis of (−)-trachyspic acid (*ent*-**1**).

Base induced elimination by adapting the method of Hatakeyama *et al.*[4] would install the C9–C10 alkene and oxidation

[a]*School of Chemistry, Bio21 Institute, 30 Flemington Rd, The University of Melbourne, Victoria 3010, Australia. E-mail: masr@unimelb.edu.au; Fax: +61 3 9347 5180; Tel: +61 3 8344 2397*
[b]*Progen Pharmaceuticals Ltd, PO Box 2403, Toowong, Queensland 4066, Australia*

† Electronic supplementary information (ESI) available: Experimental for preparation of (+)-**1**, **2**, **16**, **20**, (+)-**25**, **28** and *ent*-**28**, NMR comparison tables and copies of the ^{1}H and ^{13}C NMR spectra of compounds **1**, **23**, **25**, **28** and **31**. See DOI: 10.1039/b708594j



Scheme 2 *Reagents and conditions*: (a) NaH, PMBCl; (b) 10% HCl, MeOH; (c) NaOCl, $NaClO_2$, TEMPO; (d) DCC, DMAP, allyl alcohol; (e) (1) TMSCl–NEt_3, LDA, THF–HMPA, −95 °C, (2) aq. NaOH, (3) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea; (f) (1) 10% HCl, (2) PCC; (g) (1) DDQ, (2) MsCl, pyridine; (h) CuI, vinylMgBr, Me_2S, −45 °C; (i) (1) O_3, Me_2S, (2) $NaClO_2$, NaH_2PO_4, (3) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea.

followed by deprotection of the *t*-butyl ester would give *ent*-**1**. Lactol **2** might be available by addition of the anion derived from vinyl bromide **4** to the lactone **3** which could be obtained from the 2-deoxy-D-ribose derivative **5**. An Ireland–Claisen rearrangement in the presence of a β-leaving group[8,9] conducted on **5** would introduce the C3 stereocentre in a controlled fashion whilst the bromide **4** could be secured from dimethyl malonate (**6**). We have utilised the Ireland–Claisen rearrangement in the presence of a β-leaving group for the synthesis of the 2-alkylcitrate moiety in a number of related natural products including the cintatrins B,[10] C_1 and C_3[11] and zaragozic acid C.[12]

The synthesis of the lactone **3** is detailed in Scheme 2 and began with protection of the known alcohol in **7**[13] as the *p*-methoxybenzyl (PMB) ether followed by acid hydrolysis to give the α-anomer and β-anomer **8** in good yield. In practice, either anomer could be utilised since the anomeric centre is oxidised at a later stage in the synthesis however, we elected to continue with the β-anomer since better yields were obtained in subsequent steps. Oxidation[14] of the alcohol **8** to the acid proceeded well with only a small amount of over-oxidation of the PMB group to the benzoyl ester. This byproduct was easily removed in the next step. Esterification of the crude acid mixture with allyl alcohol then gave the pure Claisen precursor **5** after flash chromatography.

Ireland–Claisen rearrangement of **5** using our previously optimised conditions[8,10–12] followed by hydrolysis and esterification[15] gave the *t*-butyl ester **9** as the only detectable isomer in good yield. The stereochemistry of **9** was confirmed by a strong NOE interaction between H4 and the H2 protons as indicated. This showed that the [3,3]-rearrangement had occurred exclusively from the β-face opposite the OPMB group. We next investigated the introduction of the C4 vinyl group *via* a conjugate addition approach.[16] At the onset, we wanted to access both epimers as the relative configuration was, at the time, undetermined. Thus, the Claisen adduct was subjected to acid hydrolysis to afford a lactol which was oxidised to the lactone **10**. Removal of the PMB group followed by mesylation and concomitant base induced elimination gave the α,β-unsaturated lactone **11** ready for conjugate addition. Treatment of **11** with vinylmagnesium bromide in the presence of CuI and Me_2S[17] afforded the two alkene isomers **12** and **3** with a slight preference for the isomer **12** with what turned out to be the incorrect relative stereochemistry. This was confirmed by the conversion of **3** into the crystalline tri-*tert*-butylester **13** by double ozonolysis, oxidation and ester formation. A single crystal X-ray structure of **13** was determined[18] (Fig. 2) which served to confirm the structure of the minor conjugate addition diastereoisomer **3**.

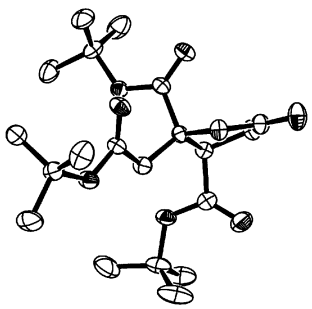
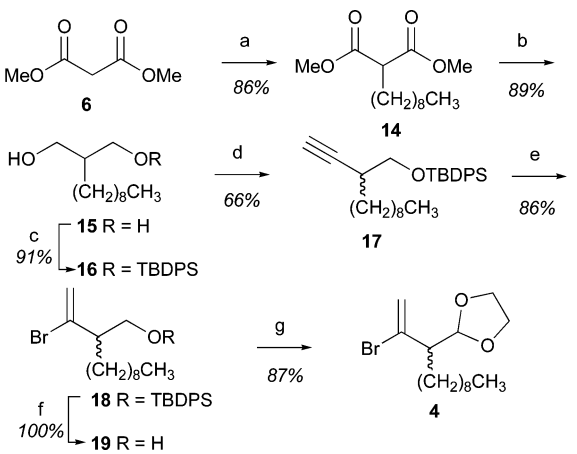
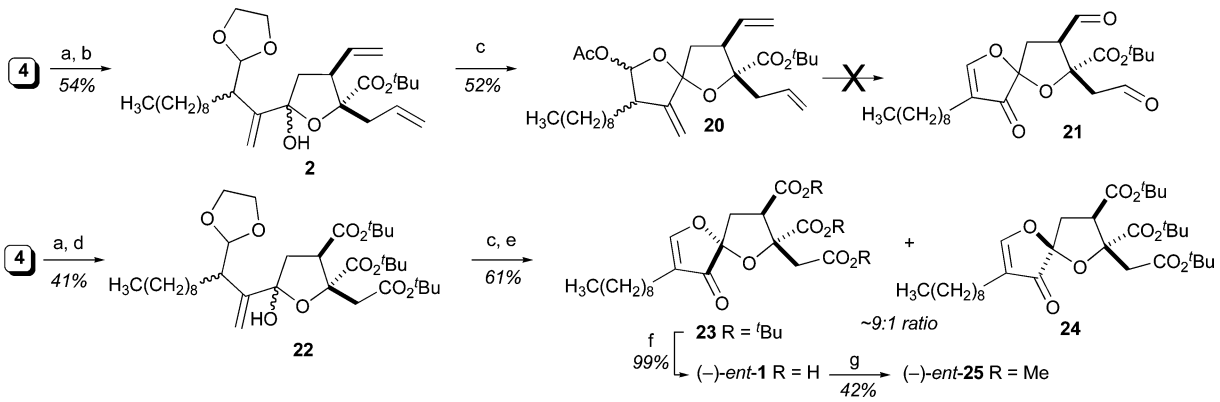


Fig. 2 X-Ray structure of ester **13** (20% ellipsoids; H atoms omitted).

The synthesis of the requisite vinyl bromide coupling partner **4** is outlined in Scheme 3. Alkylation of dimethylmalonate with nonyl bromide gave the diester **14**[19] in excellent yield. Reduction to the diol **15** was effected with $LiAlH_4$ and monoprotection[20] gave the *tert*-butyldiphenylsilyl (TBDPS) ether **16**. Oxidation of the primary alcohol in **16** and Corey–Fuchs extension[21] yielded alkyne **17**. Bromoboration[22] of **17** followed by treatment with acetic acid



Scheme 3 *Reagents and conditions*: (a) NaOMe, nonyl bromide, MeOH, reflux; (b) $LiAlH_4$, 0 °C; (c) NaH, TBDPSCl; (d) (1) Dess–Martin reagent, (2) PPh_3, CBr_4, 0 °C, (3) BuLi, −78 °C; (e) *B*-Br-9-BBN, AcOH, 0 °C; (f) TBAF; (g) (1) Dess–Martin reagent, (2) *p*-TsOH, $HOCH_2CH_2OH$, benzene, reflux.



Scheme 4 *Reagents and conditions*: (a) *t*-BuLi, Et_2O–hexane, −78 °C; (b) lactone **3**, THF; (c) (1) 3 M $HClO_4$, THF, (2) Ac_2O, DMAP, pyridine; (d) lactone **13**, THF; (e) O_3, $NaHCO_3$, Me_2S; (f) TFA, CH_2Cl_2; (g) CH_2N_2, Et_2O.

gave bromoalkene **18** which was desilylated to afford alcohol **19**. Finally, oxidation of **19** followed by acetal formation gave the dioxolane **4**.

With the two fragments in hand we next examined the coupling reaction (Scheme 4). Bromide **4** was treated with *t*-BuLi[23] in ether–hexane solvent and the resultant anion smoothly added to the lactone **3** to give the hemiacetal **2** as a mixture of isomers. This compound was then subjected to acid hydrolysis and acetylation[4] to give a complex mixture of 5,5-spiroketal diastereoisomers **20** characterised by the presence of four acetate methyl signals and one spiro carbon signal in its ^{13}C NMR spectrum. Unfortunately, all attempts at oxidative cleavage of the three alkenes in the presence of base did not afford the desired product.

At this point we envisaged that the lactone **13** may serve as an alternative coupling partner. Although there are several carbonyl groups present in **13**, we reasoned the lactone carbonyl would be the most reactive. Treatment of lactone **13** with the anion derived from **4** afforded the lactols **22** in reasonable yield along with some starting lactone **13** (48% based on recovered **13**). Acid induced cyclisation and acetylation of **22** followed by ozonolysis in the presence of $NaHCO_3$[4,7] installed the desired α,β-unsaturated system and afforded the spiroketal isomers **23** and **24** in a ratio of ~9 : 1 which were separable by flash chromatography.

The stereochemical outcome of the cyclisation can be rationalised by considering the two possible modes of attack on the oxonium ion intermediate as shown in Fig. 3.[4] Cyclisation from the least hindered α-face as in intermediate **I** affords the major isomer **23**. On the other hand, cyclisation from the β-face is not preferred as there is a larger steric interaction present as indicated in intermediate **II**. It is also not unreasonable to suggest that the cyclisation could also take place under thermodynamic control however, since acid treatment of **23** to afford *ent*-**1** (see below) did not result in any spiroisomerisation, it is most likely that the cyclisation is under kinetic control.

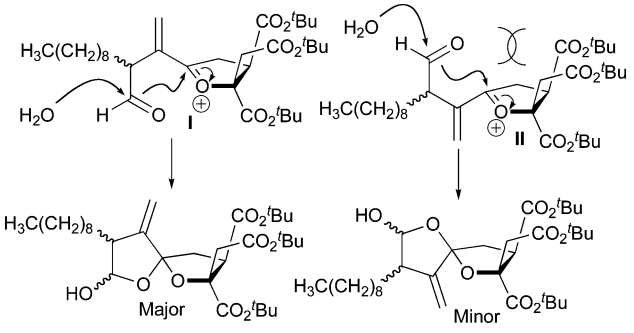


Fig. 3 Proposed spirocyclisation of intermediates **I** and **II**.

Treatment of spiroketal **23** with TFA in dichloromethane then gave (−)-*ent*-**1** which was identical to natural **1** in all respects except for the sign of optical rotation ($[\alpha]_D$ −3.5 (*c* 0.213, MeOH); $[\alpha]_D$ −8.4 (*c* 0.35, CH_2Cl_2); lit.[3] $[\alpha]_D$ +3.1 (*c* 1.0, MeOH)). This led to the assignment of the absolute configuration of (+)-trachyspic acid as (3*S*,4*S*,6*S*) shown in Fig. 1. Synthetic *ent*-**1** was further characterised by conversion into the trimethyl ester (−)-*ent*-**25** ($[\alpha]_D$ −20.4 (*c* 0.13, CH_2Cl_2) the physical data of which also compared well to those reported for naturally derived **25**. Unfortunately, the optical rotation for naturally derived **25** was not reported.[3]

For the synthesis of the correct enantiomer (+)-**1**, the lactone *ent*-**13** would be required. We envisaged that this would be available from the same deoxy-D-ribose derivative **7** utilised for the synthesis of **13**. As observed earlier, it is the stereochemistry at C4 (trachyspic acid numbering) that controls the outcome of the Ireland–Claisen rearrangement and thus the stereochemistry at C3. Therefore, if the C4 stereogenic centre is inverted as in precursor **26** (Fig. 4), this would allow for the introduction of the 3*R* stereochemistry and provide the ester required for the production of natural (+)-trachypsic acid (**1**).

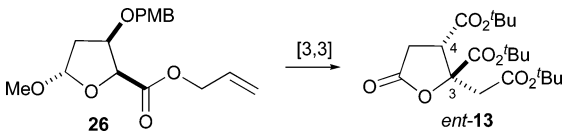
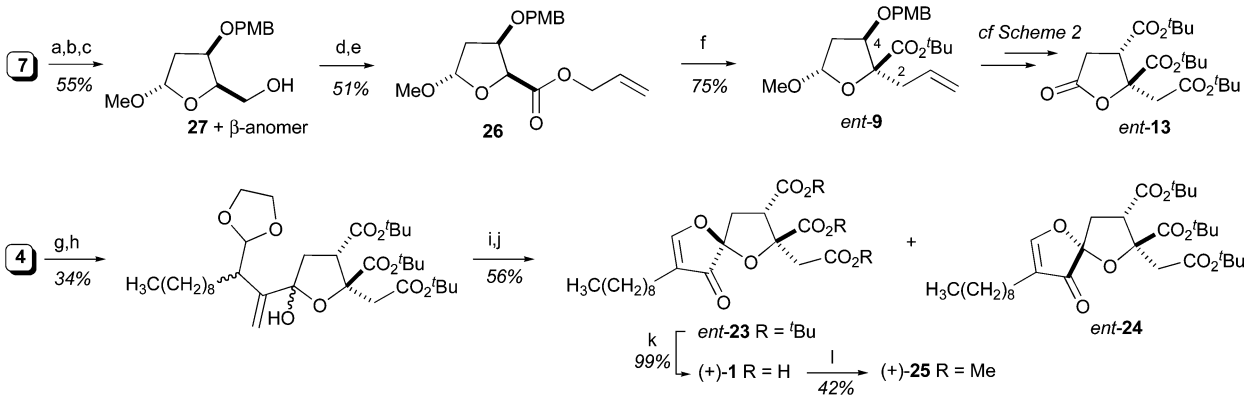


Fig. 4 Proposed synthesis of *ent*-**13**.

The conversion of the above theory into practice is outlined in Scheme 5. 2-Deoxy-D-ribose derivative **7** was subjected to a modified Mitsunobu inversion[24] and subsequent methanolysis, benzylation and trityl group hydrolysis afforded **27** along with the corresponding β-anomer. Oxidation of **27** was best achieved using a two step protocol rather than the one pot procedure utilised previously. Esterification then gave allyl ester **26** which was subjected to Ireland–Claisen rearrangement and esterification to



Scheme 5 *Reagents and conditions*: (a) (1) Ph_3P, diisopropyl azodicarboxylate (DIAD), *p*-$NO_2C_6H_4CO_2H$, (2) K_2CO_3, MeOH; (b) NaH, PMBCl; (c) 10% HCl–MeOH; (d) (1) Dess–Martin reagent, (2) Ag_2O, KOH; (e) DCC, DMAP, allyl alcohol; (f) (1) TMSCl–NEt_3, LDA, THF–HMPA, −95 °C, (2) aq. NaOH, (3) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea; (g) *t*-BuLi, Et_2O–hexane, −78 °C; (h) lactone *ent*-**13**, THF; (i) (1) 3 M $HClO_4$, THF, (2) Ac_2O, DMAP, pyridine; (j) O_3, $NaHCO_3$, Me_2S; (k) TFA, CH_2Cl_2; (l) CH_2N_2, Et_2O.

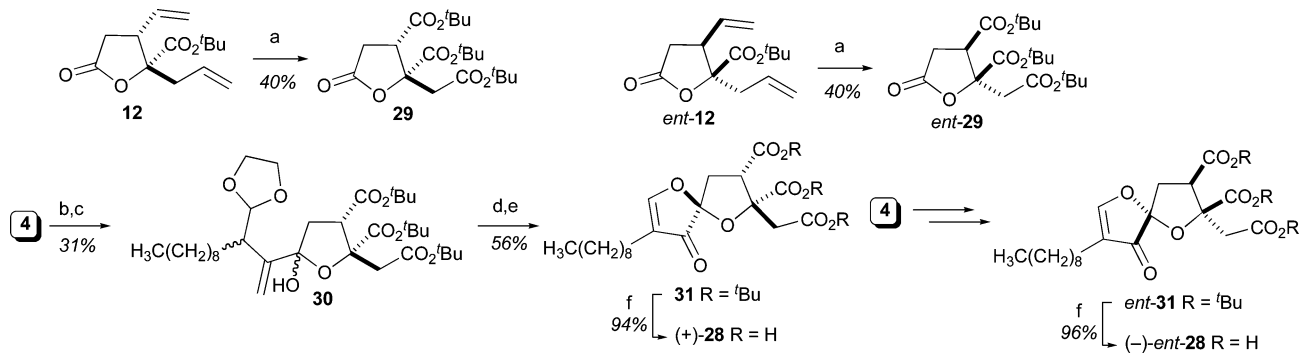
give *ent*-**9**. This was then carried through the same sequence as for **9** (see Scheme 2) to eventually give *ent*-**13** in a comparable overall yield. Addition of the anion derived from **4** to *ent*-**13** gave a mixture of lactols which underwent acid induced cyclisation and ozonolysis to give the spiroketals *ent*-**23** and *ent*-**24**. Deprotection of *ent*-**23** with TFA afforded (+)-trachyspic acid (**1**) which now had chiroptical properties that matched the natural product ($[\alpha]_D$ +3.1 (*c* 0.30, MeOH); $[\alpha]_D$ +7.1 (*c* 0.30, CH_2Cl_2); lit.[3] $[\alpha]_D$ +3.1 (*c* 1.0, MeOH)). In addition, this was further characterised by conversion into the trimethyl ester (+)-**25** ($[\alpha]_D$ +17.3 (*c* 0.10, CH_2Cl_2)).

Since the C4 isomers **12** and *ent*-**12** were also produced in the conjugate addition, we elected to carry each one though to provide (+)-3-*epi*-trachyspic acid (**28**) and its enantiomer (−)-*ent*-**28** for biological testing (Scheme 6). Ozonolysis of **12** proved troublesome with the formation of a dimethyl acetal byproduct. However, this could be removed by chromatography and the tri-*t*-butyl ester **29** and its enantiomer *ent*-**29** could be produced in moderate yield. Coupling of the anion derived from **4** with **29** afforded the lactols **30** which spirocyclised under acidic conditions. Ozonolysis then afforded triester **31** as the major compound with a trace of the spiroisomer. In this case, cyclisation occurs from the β-face of the oxonium ion (*cf.* Fig. 3) almost exclusively to give the 3*R*,4*S*,6*S* stereochemistry, epimeric at C3 compared to the natural (+)-trachyspic acid (**1**) configuration. Deprotection then afforded (+)-3-*epi*-trachyspic acid (**28**) ($[\alpha]_D^{22}$ +2.9 (*c* 0.10, MeOH)) and in a similar manner, *ent*-**29** was converted into (−)-3-*epi*-trachyspic acid (*ent*-**28**) ($[\alpha]_D^{24}$ −3.4 (*c* 0.25, MeOH)).

Synthetic (+)-trachyspic acid (**1**) and (−)-*ent*-**1** as well as (+)-3-*epi*-trachyspic acid (**28**) and (−)-*ent*-**28** were all tested for inhibition of human platelet heparanase and the results are shown in Table 1. The assays were performed using a Microcon ultrafiltration assay[25] which uses [^{3}H]-labelled HS as substrate and an ultrafiltration device to separate longer native HS from the smaller, cleaved products of heparanase catalysis which are then quantified by scintillation counting. Synthetic (+)-trachyspic acid (**1**) had a comparable IC_{50} value to that obtained for the natural product by an alternative heparanase assay.[3] Interestingly, all other stereoisomers had comparable activities with (−)-*ent*-**28** being the most active. Thus, it appears the stereochemistry is not critical for heparanase inhibition.

Table 1 Inhibition of heparanase activity

Compound	IC_{50}/μM
(+)-**1**	33.5 ± 6.5
(−)-*ent*-**1**	26.5 ± 5.4
(+)-**28**	31.7 ± 5.6
(−)-*ent*-**28**	24.5 ± 5.4



Scheme 6 *Reagents and conditions*: (a) (1) O_3, Me_2S, (2) $NaClO_2$, NaH_2PO_4, (3) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea; (b) *t*-BuLi, Et_2O–hexane, −78 °C; (c) lactone **29**, THF; (d) (1) 3 M $HClO_4$, THF, (2) Ac_2O, DMAP, pyridine; (e) O_3, $NaHCO_3$, Me_2S; (f) TFA, CH_2Cl_2.

Conclusions

The synthesis of both natural (+)-trachyspic acid (**1**) and its enantiomer (−)-*ent*-**1** as well as both enantiomers of 3-*epi*-trachyspic acid (**28** and *ent*-**28**) has been achieved from a common chiral precursor **7** derived from 2-deoxy-D-ribose. The key steps in the sequences used are a stereoselective Ireland–Claisen rearrangement in the presence of a β-leaving group, a selective carbanion addition to a lactone tri-*t*-butylester and a spirocyclisation–ozonolysis–elimination sequence to construct the spirolactone system in a stereoselective manner. The activity against heparanase of all four stereoisomers is similar indicating that the stereochemistry of the triacid is not critical for activity against this target.

Experimental

General

Optical rotations were recorded in a 10 cm microcell. High resolution mass spectra (HRMS) were run using electrospray ionisation (ESI). Proton nuclear magnetic resonance (^{1}H NMR, 300, 400 and 500 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 75.5, 100 and 125 MHz) were recorded for deuteriochloroform solutions with residual chloroform as internal standard unless otherwise stated. Analytical thin layer chromatography (TLC) was conducted on aluminium backed 2 mm thick silica gel GF_{254}. Compounds were visualised with solutions of 20% w/w phosphomolybdic acid in ethanol, 20% w/w potassium permanganate in water or under UV (365 nm). Anhydrous tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl and sodium metal under a nitrogen atmosphere. Petrol refers to the fraction boiling at 40–60 °C. All other commercial reagents were used as received. All air and moisture sensitive reactions were performed in glassware that was either flame dried under an atmosphere of dry argon or oven dried at 150 °C.

Methyl furanoside 8

A solution of commercial 2-deoxy-D-ribose (14.8 g, 0.110 mol) in anhydrous MeOH (235 cm^3) was cooled to 0 °C and treated with concentrated H_2SO_4 (5 cm^3). The solution was stirred at 0 °C for 1 h and then neutralised with solid NH_4HCO_3. The suspension was filtered and the filtrate was concentrated. The crude residue was dissolved in *N*,*N*-dimethylformamide (DMF, 140 cm^3) and pyridine (19.2 cm^3), 4-(dimethylamino)pyridine (DMAP, 1.34 g, 0.011 mol) and trityl chloride (61.3 g, 0.220 mol) were added. The solution was stirred at rt for 16 h and saturated aqueous $NaHCO_3$ was added and the aqueous phase was extracted with Et_2O. The combined organic extracts were washed with saturated aqueous $CuSO_4$, water then brine, dried and concentrated. Purification by flash chromatography with 10–40% EtOAc–petrol as eluent gave alcohol **7** (37.4 g, 87%) as a yellow gum. A solution of **7** (37.4 g, 0.0993 mol) in DMF (250 cm^3) was added *via* cannula to a suspension of 60% NaH dispersion in oil (4.77 g, 0.119 mol) in DMF (200 cm^3). To the suspension was added *p*-methoxybenzyl chloride (PMBCl, 16.2 cm^3, 0.119 mol) and the reaction mixture was stirred at rt for 16 h. The reaction was quenched with water and the organic phase was extracted with Et_2O. The combined organic extracts were washed with water then brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude residue was dissolved in MeOH (1000 cm^3) and treated with 10% HCl (1 cm^3). The reaction was stirred at rt overnight and quenched with saturated aqueous $NaHCO_3$. The MeOH was removed under reduced pressure and the aqueous phase was extracted with Et_2O, washed with water then brine, and dried. The crude product was purified by column chromatography with 20% EtOAc–petrol as eluent to afford the β-anomer **8** (9.22 g, 36% for 2 steps) as a yellow oil: $[\alpha]_D^{14}$ −38.4 (*c* 1.07, CH_2Cl_2); ν_{max} (film) 3447, 2937, 2836, 1613, 1514, 1037 cm^{-1}; δ_H (400 MHz) 2.08 (br s, 1H), 2.16–2.27 (m, 2H), 3.39 (s, 3H), 3.57 (dd, J = 12.0, 3.6 Hz, 1H), 3.73 (dd, J = 12.0, 2.6 Hz, 1H), 3.80 (s, 3H), 4.26 (m, 2H), 4.42 (s, 2H), 5.12 (dd, J = 5.6, 2.6 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H); δ_C (100 MHz, $CDCl_3$) 40.2, 55.2, 55.4, 64.0, 71.4, 78.6, 85.7, 105.7, 113.8, 129.3, 129.8, 159.3; HRMS (ESI): calculated for $C_{14}H_{20}O_5Na$ [M + Na]$^+$ 291.1208, found 291.1205.

Further elution with 40% EtOAc–petrol afforded the α-anomer (11.7 g, 44%) as a yellow gum: $[\alpha]_D^{14}$ +139.5 (*c* 1.04, CH_2Cl_2); ν_{max} 3461, 2911, 2837, 1613, 1514, 1037 cm^{-1}; δ_H (400 MHz) 1.73 (br s, 1H), 1.98 (dd, J = 13.9, 1.6 Hz, 1H), 2.18 (ddd, J = 13.9, 8.0, 4.8 Hz, 1H), 3.37 (s, 3H), 3.52–3.74 (m, 2H), 3.77 (s, 3H), 3.95 (m, 1H), 4.09 (dd, J = 8.0, 4.4 Hz, 1H), 4.45 (ABq, J = 12.0 Hz, 2H), 5.02 (d, J = 4.8 Hz, 1H), 6.86 (d, J = 8.8 Hz, 2H), 7.24 (d, J = 8.8 Hz, 2H); δ_C (100 MHz) 39.3, 55.1, 55.2, 62.7, 71.5, 77.6, 83.0, 105.2, 113.8, 129.4, 130.0, 159.2; HRMS (ESI): calculated for $C_{14}H_{20}O_5Na$ [M + Na]$^+$ 291.1208, found 291.1204.

Allyl ester 5

A solution of alcohol **8** (2.83 g, 10.5 mmol) and tetramethylpiperidine-*N*-oxyl (TEMPO, 115 mg, 0.738 mmol) in acetonitrile (52 cm^3) and pH 7 buffer (41 cm^3) was warmed to 35 °C and solutions of 80% $NaClO_2$ (2.39 g, 21.0 mmol) in water (10.5 cm^3) and 8% NaOCl (196 μL, 0.211 mmol) in water (5.2 cm^3) were then added simultaneously to the reaction mixture over 15 min. The reaction mixture was stirred at 35 °C for 24 h and then cooled to rt. Water (75 cm^3) was added and the pH was adjusted to 8 using 1 M aqueous NaOH. The mixture was cooled to 0 °C and a solution of Na_2SO_3 (3.15 g) in water (49 cm^3) was added. The solution was warmed to rt and stirred for 30 min. Et_2O was added and the organic phase was separated and concentrated under reduced pressure to recover starting alcohol **8** (0.62 g). The aqueous layer was acidified with 10% HCl and the aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water then brine, and dried. The solvent was removed under reduced pressure and the crude residue was dissolved in dry CH_2Cl_2 (45 cm^3), cooled to 0 °C and DMAP (90 mg, 0.737 mmol), allyl alcohol (551 μL, 8.11 mmol) and 1,3-dicyclohexylcarbodiimide (DCC, 1.60 g, 7.74 mmol) were added. The mixture was stirred for 1 h at 0 °C then warmed to rt and stirred for another 1 h. The white suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography with 10% EtOAc–petrol as eluent to give ester **5** (1.98 g, 75% for 2 steps) as a pale yellow oil: $[\alpha]_D^{13}$ −58.8 (*c* 1.05, CH_2Cl_2); ν_{max} (thin film) 2934, 2836, 1756, 1613, 1250 cm^{-1}; δ_H (400 MHz) 2.06 (m, 1H), 2.21 (m, 1H), 3.36 (s, 3H), 3.77 (s, 3H), 4.46 (ABq, J = 11.6 Hz,

1H), 4.52 (ABq, $J = 11.6$ Hz, 1H), 4.53 (m, 1H), 4.55 (m, 2H), 4.64 (d, $J = 6.0$ Hz, 1H), 5.13 (dd, $J = 5.2$, 1.2 Hz, 1H), 5.24 (d, $J = 10.6$ Hz, 1H), 5.33 (dd, $J = 17.2$, 1.2 Hz, 1H), 5.91 (ddd, $J = 17.2$, 10.6, 5.6 Hz, 1H), 6.84 (d, $J = 8.6$ Hz, 2H), 7.24 (d, $J = 8.6$ Hz, 2H); δ_C (100 MHz) 39.7, 55.2, 55.3, 65.9, 71.7, 80.6, 81.9, 106.2, 113.8, 118.8, 129.4, 129.6, 131.6, 159.3, 171.3; HRMS (ESI): calculated for $C_{17}H_{22}O_6Na$ $[M + Na]^+$ 345.1314, found 345.1313.

tert-Butyl ester 9

A solution of ^{n}BuLi in hexanes (3.4 cm^3, 2.3 M, 7.71 mmol) was added dropwise to a solution of $^i Pr_2NH$ (0.98 cm^3, 7.01 mmol) in dry THF (17 cm^3) at 0 °C under argon. The resultant lithium diisopropylamide (LDA) solution was stirred at 0 °C for 5 min, cooled to −78 °C and added dropwise *via* cannula to a solution of the allyl ester **5** (1.13 g, 3.51 mmol), hexamethylphosphoramide (HMPA, 4.6 cm^3) and the supernatant from a centrifuged mixture of freshly distilled trimethylsilyl chloride (TMSCl, 2.5 cm^3, 19.3 mmol) and NEt_3 (2.5 cm^3, 17.5 mmol) in dry THF (26 cm^3) at −100 °C (liquid N_2/MeOH bath). The reaction mixture was stirred at −100 °C for 10 min and then allowed to warm to rt and left to stir for 2 h. The solution was cooled to 0 °C and aqueous 1 M NaOH (25 cm^3) was added followed by Et_2O and water. The organic phase was separated and discarded and the aqueous phase was then acidified with 10% HCl at 0 °C and extracted with Et_2O. The combined extracts were washed with water then brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was dissolved in dry CH_2Cl_2 (50 cm^3) and treated with *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea (3.23 g, 16.1 mmol) for 16 h at rt. The white suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography with 10% EtOAc–petrol as eluent to give the *tert*-butyl ester **9** (1.02 g, 77% for 2 steps) as a colourless oil: $[\alpha]_D^{14}$ −55.1 (*c* 0.98, CH_2Cl_2); ν_{max} (thin film) 2979, 2837, 1733, 1614, 1515, 1249, 1036 cm^{-1}; δ_H (400 MHz) 1.43 (s, 9H), 2.11 (ddd, $J = 13.1$, 6.4, 1.6 Hz, 1H), 2.28 (m, 1H), 2.46 (dd, $J = 14.2$, 7.2 Hz, 1H), 2.81 (dd, $J = 14.2$, 7.2 Hz, 1H), 3.37 (s, 3H), 3.80 (s, 3H), 4.13 (t, $J = 6.8$ Hz, 1H), 4.44 (ABq, $J = 11.6$ Hz, 1H), 4.52 (ABq, $J = 11.6$ Hz, 1H), 5.10 (s, 1H), 5.13 (d, $J = 7.2$ Hz, 1H), 5.21 (dd, $J = 5.6$, 1.6 Hz, 1H), 5.86 (ddt, $J = 17.2$, 10.0, 7.2 Hz, 1H), 6.85 (d, $J = 8.6$ Hz, 2H), 7.22 (d, $J = 8.6$ Hz, 2H); δ_C (100 MHz) 28.0, 38.3, 41.9, 55.2, 55.3, 71.9, 81.5, 82.7, 88.6, 104.8, 113.6, 118.3, 129.2, 129.8, 133.1, 159.1, 169.8; HRMS (ESI): calculated for $C_{21}H_{30}O_6Na$ $[M + Na]^+$ 401.1940, found 401.1936.

Lactone 10

To a solution of the ester **9** (1.02 g, 2.70 mmol) in THF (50 cm^3) was added 10% aqueous HCl (35 cm^3) and the reaction was stirred for 40 h at rt and diluted with water and Et_2O. The aqueous phase was extracted with Et_2O, washed with water then brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure and the resulting lactol mixture was dissolved in dry CH_2Cl_2 (60 cm^3) and 4 Å sieves (1.54 g) were added. The suspension was cooled to 0 °C and pyridinium chlorochromate (PCC, 880 mg, 4.04 mmol) was added. The reaction was stirred for 2 h at 0 °C, warmed to rt and stirred overnight. The brown suspension was filtered through Fluorosil, washed with large quantities of Et_2O and the solvent was removed under reduced pressure. The remaining dark coloured residue was purified by column chromatography with 20% EtOAc–petrol as eluent to give the lactone **10** (820 mg, 84% for 2 steps) as a pale yellow oil: $[\alpha]_D^{20}$ +3.8 (*c* 1.09, CH_2Cl_2); ν_{max} (thin film) 2934, 1756, 1613, 1514, 1037 cm^{-1}; δ_H (400 MHz) 1.45 (s, 9H), 2.53 (dd, $J = 14.8$, 7.2 Hz, 1H), 2.69 (d, $J = 6.8$ Hz, 2H), 2.84 (dd, $J = 14.8$, 7.2 Hz, 1H), 3.80 (s, 3H), 4.16 (t, $J = 7.2$ Hz, 1H), 4.46 (ABq, $J = 11.6$ Hz, 1H), 4.52 (ABq, $J = 11.6$ Hz, 1H), 5.15 (m, 2H), 5.73 (ddt, $J = 16.8$, 10.0, 7.2 Hz, 1H), 6.86 (d, $J = 8.6$ Hz, 2H), 7.20 (d, $J = 8.6$ Hz, 2H); δ_C (100 MHz) 27.9, 34.9, 39.3, 55.2, 72.3, 77.9, 83.1, 88.4, 113.8, 120.4, 128.7, 129.4, 130.7, 159.5, 167.3, 173.4; HRMS (ESI): calculated for $C_{20}H_{26}O_6Na$ $[M + Na]^+$ 385.1627, found 385.1617.

α,β-Unsaturated lactone 11

To a solution of the lactone **10** (820 mg, 2.26 mmol) in CH_2Cl_2 (100 cm^3) and water (5.6 cm^3) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1.28 g, 5.66 mmol). The biphasic suspension was stirred at rt for 16 h and the crude reaction mixture was filtered through Celite and the filtrate was concentrated. The crude product was purified by column chromatography with 30% EtOAc–petrol as eluent to give an alcohol (507 mg, 92%) as a pale pink oil: $[\alpha]_D^{18}$ +13.5 (*c* 1.01, CH_2Cl_2); ν_{max} (thin film) 3466, 2983, 1789, 1736, 1267, 1161 cm^{-1}; δ_H (400 MHz) 1.47 (s, 9H), 2.47 (dd, $J = 14.4$, 7.0 Hz, 1H), 2.65 (dd, $J = 18.2$, 4.4 Hz, 1H), 2.74 (dd, $J = 14.4$, 7.0 Hz, 1H), 2.84 (dd, $J = 18.2$, 7.4 Hz, 1H), 3.34 (br s, 1H), 4.47 (dd, $J = 7.4$, 4.4 Hz, 1H), 5.16 (s, 1H), 5.19 (d, $J = 8.8$ Hz, 1H), 5.72 (ddt, $J = 17.2$, 10.0, 7.0 Hz, 1H); δ_C (100 MHz) 27.9, 36.9, 39.5, 72.1, 83.8, 90.2, 120.5, 130.2, 167.8, 174.3; HRMS (ESI): calculated for $C_{12}H_{18}O_5Na$ $[M + Na]^+$ 265.1052, found 265.1047. A solution of the alcohol (507 mg, 2.09 mmol) in pyridine (20 cm^3) was cooled to 0 °C. The solution was treated with MsCl (486 μL, 6.28 mmol) at 0 °C for 2 h and then warmed to rt and stirred overnight. Water and Et_2O were added and the aqueous phase was extracted further with Et_2O. The combined organic extracts were washed with saturated aqueous $CuSO_4$, water, then brine, dried and concentrated under reduced pressure. The crude residue was purified by column chromatography, eluting with 15–20% EtOAc–petrol to afford the α,β-unsaturated lactone **11** (423 mg, 90%) as a pale yellow low melting crystalline solid: $[\alpha]_D^{13}$ +161.8 (*c* 1.01, CH_2Cl_2); ν_{max} (thin film) 2983, 1772, 1372, 1258, 1133 cm^{-1}; δ_H (400 MHz) 1.45 (s, 9H), 2.64 (dd, $J = 14.4$, 6.8 Hz, 1H), 2.84 (dd, $J = 14.4$, 7.6 Hz, 1H), 5.17 (s, 1H), 5.20 (d, $J = 4.8$ Hz, 1H), 5.67 (m, 1H), 6.13 (d, $J = 5.8$ Hz, 1H), 7.39 (d, $J = 5.8$ Hz, 1H); δ_C (100 MHz) 27.8, 39.8, 84.0, 89.5, 120.9, 122.2, 129.4, 154.6, 165.9, 171.5; HRMS (ESI): calculated for $C_{12}H_{16}O_4Na$ $[M + Na]^+$ 247.0946, found 247.0944.

Dienes 12 and 3

To a flame dried flask containing CuI (395 mg, 2.07 mmol) was added dry THF (10 cm^3) under argon. The pale brown suspension was cooled to −78 °C and a solution of vinylMgBr in THF (4.6 cm^3, 0.9 M, 4.15 mmol) was added. The resulting yellow suspension was stirred for 10 min at −78 °C and Me_2S (102 μL, 1.38 mmol) was then added and the suspension was stirred at −78 °C for 90 min. A solution of the lactone **11** (155 mg, 0.691 mmol) in dry THF (4.5 cm^3) was then added *via* cannula.

The reaction mixture was immediately warmed to −45 °C and stirred for 1 h then quenched with saturated aqueous NH_4Cl and diluted with Et_2O. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water then brine, dried and concentrated. The crude product was purified by column chromatography with 80% CH_2Cl_2–petrol as eluent to afford lactone **12** (80 mg, 46%) as a colourless oil: $[\alpha]_D^{23}$ −24.8 (*c* 1.03, CH_2Cl_2); ν_{max} (thin film) 2981, 1796, 1733, 1370, 1252, 1152 cm^{-1}; δ_H (400 MHz) 1.45 (s, 9H), 2.51 (dd, $J = 14.8$, 8.0 Hz, 1H), 2.58 (dd, $J = 17.2$, 8.8 Hz, 1H), 2.67 (dd, $J = 17.2$, 11.2 Hz, 1H), 2.86 (dd, $J = 14.8$, 6.4 Hz, 1H), 3.12 (dd, $J = 17.8$, 7.6 Hz, 1H), 5.16–5.23 (m, 4H), 5.68 (ddd, $J = 17.2$, 10.4, 7.6 Hz, 1H), 5.79 (m, 1H); δ_C (100 MHz) 28.0, 33.2, 38.7, 47.2, 83.6, 87.8, 119.3, 120.4, 131.1, 132.5, 168.1, 174.9; HRMS (ESI): calculated for $C_{14}H_{20}O_4Na$ $[M + Na]^+$ 275.1259, found 275.1257.

Further elution gave the C4 epimer **3** (56 mg, 32%) as a pale yellow oil: $[\alpha]_D^{22}$ +18.1 (*c* 0.87, CH_2Cl_2); ν_{max} (thin film) 2981, 1791, 1732, 1370, 1132 cm^{-1}; δ_H (400 MHz) 1.47 (s, 9H), 2.42 (dd, $J =$ 14.4, 6.0 Hz, 1H), 2.45 (dd, $J = 17.6$, 6.0 Hz, 1H), 2.58 (dd, $J =$ 14.4, 8.0 Hz, 1H), 2.73 (dd, $J = 18.0$, 8.4 Hz, 1H), 3.23 (dd, $J =$ 14.4, 8.4 Hz, 1H), 5.14–5.29 (m, 4H), 5.72–5.86 (m, 2H); δ_C (100 MHz) 27.9, 33.7, 37.9, 46.4, 83.2, 87.9, 119.1, 120.0, 130.9, 133.2, 169.5, 174.5; HRMS (ESI): calculated for $C_{14}H_{20}O_4Na$ $[M + Na]^+$ 275.1259, found 275.1260.

Lactone triester 13

Ozone gas was bubbled through a solution of the diene **3** (30 mg, 0.119 mmol) in CH_2Cl_2 (3.0 cm^3) and MeOH (180 μL) at −78 °C until a pale blue colour persisted. Me_2S (87 μL, 1.19 mmol) was added and the solution was allowed to warm to rt and stirring was continued for a further 4 h. Water and Et_2O were added and the aqueous phase was extracted with Et_2O. The combined extracts were washed with water then brine, and dried over $MgSO_4$ and concentrated under reduced pressure. The crude dialdehyde was dissolved in *tert*-butanol (3.0 cm^3) and treated with 2-methyl-2-butene (500 μL). A solution of $NaH_2PO_4 \cdot H_2O$ (131 mg, 0.951 mmol) and 80% $NaClO_2$ (215 mg, 1.90 mmol) in water (1.6 cm^3) was then added and the reaction mixture was stirred at rt for 16 h. Water and Et_2O were added and the organic layer was separated and discarded. The aqueous phase was acidified with 10% HCl and extracted with Et_2O and the combined extracts were washed with water then brine, and dried. The solvent was removed under reduced pressure and the residue was dissolved in dry CH_2Cl_2 (9.5 cm^3) and treated with *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea (476 mg, 2.38 mmol) for 16 h at rt. The suspension was filtered through Celite and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography with 10% EtOAc–petrol as eluent to give the triester **13** (30 mg, 63%) as a colourless crystalline solid; $[\alpha]_D^{14}$ −15.5 (*c* 0.99, CH_2Cl_2); ν_{max} (thin film) 2980, 1803, 1732, 1369, 1148 cm^{-1}; δ_H (400 MHz) 1.44 (s, 9H), 1.48 (s, 9H), 1.49 (s, 9H), 2.75 (dd, $J = 17.6$, 4.0 Hz, 1H), 2.83 (dd, $J = 17.6$, 8.6 Hz, 1H), 2.87 (ABq, $J = 17.2$ Hz, 1H), 3.08 (ABq, $J = 17.2$ Hz, 1H), 3.39 (dd, $J = 8.6$, 4.0 Hz, 1H); δ_C (100 MHz) 27.7, 27.9, 28.0, 32.1, 39.0, 47.6, 82.0, 83.2, 83.6, 83.7, 167.6, 168.3, 169.0, 174.0; HRMS (ESI): calculated for $C_{20}H_{32}O_8Na$ $[M + Na]^+$ 423.1995, found 423.1990.

Alkyne 17

Dess–Martin periodinane (12.51 g, 29.5 mmol) was added to a cooled solution of alcohol **16** (8.29 g, 18.8 mmol) in CH_2Cl_2 (200 cm^3) at 0 °C. The solution was warmed to rt, stirred for 30 min and quenched with saturated aqueous $NaHCO_3$ and 1.5 M $Na_2S_2O_3$. The biphasic solution was stirred for 15 min, until two clear layers formed and the aqueous phase was extracted with Et_2O. The combined organic extracts were washed with water then brine, dried and concentrated. A solution of the crude aldehyde in CH_2Cl_2 (150 cm^3) was cooled to 0 °C and PPh_3 (19.72 g, 75.2 mmol) and CBr_4 (12.47 g, 37.6 mmol) were added. The orange solution was stirred at 0 °C for 1 h and the solvent was removed under reduced pressure. The crude residue was triturated with petrol, filtered and the filtrate was concentrated to afford a yellow oil (8.73 g, 14.6 mmol) which was dissolved in dry THF (200 cm^3), cooled to −78 °C and a solution of ^{n}BuLi in hexanes (2.1 M, 14.0 cm^3, 29.4 mmol) was added dropwise. Water was added and the aqueous phase was extracted with petrol and the combined organic extracts were washed with water then brine, dried and concentrated. Purification by flash chromatography with 2.5% EtOAc–petrol as eluent gave the alkyne **17** (5.40 g, 66%) as a colourless oil: ν_{max} 2928, 2857, 1428, 1112 cm^{-1}; δ_H (400 MHz) 0.89 (t, $J = 6.8$ Hz, 3H), 1.07 (s, 9H), 1.28 (br s, 14H), 1.66 (m, 2H), 2.04 (d, $J = 2.4$ Hz, 1H), 2.56 (m, 1H), 3.61 (dd, $J = 10.0$, 7.6 Hz, 1H), 3.75 (dd, $J = 10.0$, 5.6 Hz, 1H), 7.40 (m, 5H), 7.69 (m, 5H); δ_C (100 MHz) 14.1, 19.3, 22.7, 26.8, 26.8, 29.3, 29.4, 29.5, 29.6, 30.9, 31.9, 34.5, 66.1, 69.8, 85.6, 127.6, 129.6, 133.6, 135.6, 135.6; HRMS (ESI): calculated for $C_{29}H_{42}OSiH$ $[M + H]^+$ 435.3083, found 435.3084.

Bromoalkene 18

A solution of alkyne **17** (5.40 g, 12.4 mmol) in CH_2Cl_2 (100 cm^3) was cooled to 0 °C and *B*-bromo-9-borabicyclo[3.3.1]nonane (*B*-Br-9-BBN, 1.0 M in THF, 24.8 cm^3, 24.8 mmol) was added. The reaction was stirred at 0 °C for 3 h and glacial acetic acid (11.4 cm^3, 198 mmol) was then added and the solution was stirred for 1 h. Water was added and the aqueous phase was extracted with petrol. The combined organic extracts were washed with water then brine, dried and concentrated and the crude product was purified by flash chromatography with petrol as eluent to give vinyl bromide **18** (5.51 g, 86%) as a colourless oil: ν_{max} 2928, 2856, 1428, 1113 cm^{-1}; δ_H (400 MHz) 0.89 (t, $J = 6.8$ Hz, 3H), 1.05 (s, 9H), 1.25 (br s, 14H), 1.32 (m, 2H), 2.41 (quint., $J = 6.0$ Hz, 1H), 3.55 (dd, $J =$ 10.0, 5.2 Hz, 1H), 3.66 (dd, $J = 10.0$, 8.0 Hz, 1H), 5.54 (s, 1H), 5.68 (s, 1H), 7.40 (m, 5H), 7.68 (m, 5H); δ_C (100 MHz) 14.1, 19.3, 22.7, 26.7, 26.8, 29.1, 29.3, 29.4, 29.5, 29.5, 31.9, 52.3, 65.3, 118.4, 127.6, 127.6, 129.6, 133.6, 133.8, 135.6, 135.7, 137.2; HRMS (ESI): calculated for $C_{29}H_{43}BrOSiNa$ $[M + Na]^+$ 537.2164, found 537.2169.

Acetal 4

Tetrabutylammonium fluoride trihydrate (TBAF·$3H_2O$, 8.43 g, 26.7 mmol) was added to a solution of the silyl ether **18** (5.51 g, 10.7 mmol) in THF (150 cm^3) and the solution was stirred at rt for 16 h. Water was added and the aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water then brine, dried and concentrated to afford the alcohol **19** (2.96 g,

100%) as a yellow oil: ν_{max} 3339, 2926, 2855, 1466, 1050 cm^{-1}; δ_H (400 MHz) 0.87 (t, $J = 6.8$ Hz, 3H), 1.25 (br s, 14H), 1.38 (m, 2H), 1.61 (br s, 1H), 2.41 (quint., $J = 4.4$ Hz, 1H), 3.51 (dd, $J = 11.2$, 5.2 Hz, 1H), 3.59 (dd, $J = 11.2$, 8.8 Hz, 1H), 5.59 (d, $J = 1.0$ Hz, 1H), 5.74 (d, $J = 1.0$ Hz, 1H); δ_C (100 MHz) 14.1, 22.6, 26.7, 29.1, 29.3, 29.4, 29.4, 29.5, 31.8, 52.5, 64.2, 119.7, 136.6; HRMS (ESI): calculated for $C_{13}H_{25}BrONa$ [M + Na]$^+$ 299.0986, found 299.0982. A solution of the alcohol **19** (2.96 g, 10.7 mmol) in CH_2Cl_2 (200 cm^3) was cooled to 0 °C and Dess–Martin periodinane (5.90 g, 13.9 mmol) was added. The solution was warmed to rt and stirred for 30 min, then quenched with saturated aqueous $NaHCO_3$ and 1.5 M $Na_2S_2O_3$. The biphasic solution was stirred for 15 min until two clear layers formed and the aqueous phase was extracted with Et_2O. The combined organic extracts were washed with water then brine, dried and concentrated. The crude residue was dissolved in dry benzene (150 cm^3) and *p*-TsOH (204 mg, 1.07 mmol) and ethylene glycol (6.0 cm^3, 107 mmol) were added and the solution was heated under reflux on a Dean Stark apparatus for 16 h and then cooled to rt. Saturated aqueous $NaHCO_3$ was added and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with water then brine, dried and concentrated and the crude product was purified by flash chromatography with 2.5% EtOAc–petrol as eluent to afford acetal **4** (2.96 g, 87%) as a colourless oil: ν_{max} 2925, 2855, 1466, 1120 cm^{-1}; δ_H (400 MHz) 0.87 (t, $J = 7.2$ Hz, 3H), 1.25 (br s, 14H), 1.49–1.62 (m, 2H), 2.34 (m, 1H), 3.86–3.99 (m, 4H), 4.85 (d, $J = 6.4$ Hz, 1H), 5.58 (d, $J = 1.2$ Hz, 1H), 5.72 (d, $J = 1.2$ Hz, 1H); δ_C (100 MHz) 14.1, 22.6, 26.5, 28.1, 29.3, 29.4, 29.5, 31.9, 53.8, 64.9, 65.0, 104.9, 119.5, 119.6, 133.4; HRMS (ESI): calculated for $C_{15}H_{27}BrO_2H$ [M + H]$^+$ 319.1266, found 319.1266.

Lactols 22

A solution of ^{t}BuLi in hexanes (392 μL, 1.4 M, 0.549 mmol) was added dropwise to a solution of bromoalkene **4** (100 mg, 0.313 mmol) in dry Et_2O (1.0 cm^3) and dry hexane (0.5 cm^3) at −78 °C under argon. The solution was stirred at −78 °C for 5 min and a solution of the triester **13** (44 mg, 0.110 mmol) in Et_2O (1.0 cm^3) and hexane (0.5 cm^3) was added dropwise *via* cannula to the anion solution. The reaction mixture was stirred at −78 °C for 4 h and diluted with water and Et_2O. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water then brine and dried. The solvent was removed under reduced pressure and purification by column chromatography with 10% EtOAc–petrol gave recovered triester **13** (20 mg). Further elution afforded the lactols **22** (24 mg, 41%, 62% based on recovered **13**) as a pale yellow oil: ν_{max} (thin film) 3497, 2928, 2856, 1735, 1369, 1153 cm^{-1}; δ_H (400 MHz) 0.86 (m, 3H), 1.23 (m, 14H), 1.43 (s, 9H), 1.44 (s, 9H), 1.47 (s, 9H), 1.63 (m, 2H), 2.50 (dd, $J = 18.0$, 2.8 Hz, 0.5H), 2.65 (dd, $J = 18.0$, 2.8 Hz, 0.5H), 2.80 (dd, $J = 16.8$, 7.6 Hz, 1H), 2.93 (d, $J = 17.2$ Hz, 1H), 3.05 (m, 1H), 3.39 (dd, $J = 18.0$, 11.2 Hz, 0.5H), 3.50 (dd, $J = 18.0$, 11.6 Hz, 0.5H), 3.72–3.94 (m, 5H), 4.78 (d, $J = 5.2$ Hz, 0.5H), 4.85 (d, $J = 5.2$ Hz, 0.5H), 5.81 (d, $J = 4.4$ Hz, 1H), 6.18 (d, $J = 8.0$ Hz, 1H); δ_C (100 MHz, $CDCl_3$) 14.1, 22.7, 26.9, 27.0, 27.0, 27.8, 27.9, 28.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 29.7, 31.9, 32.2, 35.8, 35.9, 42.3, 42.6, 42.6, 43.5, 48.6, 48.7, 50.2, 61.3, 64.7, 64.8, 65.0, 66.2, 75.1, 75.1, 81.4, 81.4, 83.1, 105.8, 106.3, 117.2, 125.0, 125.8, 136.0, 147.2, 147.4, 170.0, 170.2, 172.6, 199.3, 199.5; HRMS (ESI): calculated for $C_{35}H_{60}O_{10}Na$ [M + Na]$^+$ 663.4084, found 663.4083.

(−)-Trachyspic acid tri-*tert*-butyl ester 23

A solution of the lactol mixture **22** (18 mg, 0.0281 mmol) in THF (1.0 cm^3) was cooled to 0 °C and treated with 3 M $HClO_4$ (0.5 cm^3). The solution was stirred at 0 °C for 1 h, quenched with saturated aqueous $NaHCO_3$ and the aqueous phase was extracted with Et_2O, washed with water then brine and dried. The solvent was removed under reduced pressure and the residue was dissolved in pyridine (1.0 cm^3) and DMAP (0.34 mg, 0.00281 mmol) and acetic anhydride (27 μL, 0.281 mmol) were added. The solution was stirred at rt overnight and then diluted with water and Et_2O and the aqueous phase was extracted with Et_2O. The organic extracts were washed with saturated aqueous $CuSO_4$, water then brine, dried and concentrated. The crude residue was dissolved in CH_2Cl_2 (2.0 cm^3) and MeOH (200 μL) and ozone gas was bubbled through the solution at −78 °C until a pale blue colour persisted. Me_2S (21 μL, 0.281 mmol) and $NaHCO_3$ (21 mg, 0.281 mmol) were added and the solution was warmed to rt and stirred for 16 h. Water and Et_2O were added and the aqueous phase was extracted with Et_2O. The combined organic extracts were washed with water then brine, dried and concentrated. The crude residue was purified by column chromatography with 5–10% EtOAc–petrol as eluent to afford the minor spiroisomer **24** (1 mg, 6% for 3 steps) as a thin film: $[\alpha]_D^{24}$ −25.5 (*c* 0.03, CH_2Cl_2); ν_{max} (thin film) 2927, 1732, 1367, 1151 cm^{-1}; δ_H (500 MHz) 0.88 (t, $J = 8.0$ Hz, 3H), 1.25 (br s, 14H), 1.42 (s, 9H), 1.47 (s, 9H), 1.52 (s, 9H), 2.08 (t, $J = 8.0$ Hz, 2H), 2.51 (dd, $J = 13.5$, 5.5 Hz, 1H), 2.67 (dd, $J = 13.5$, 9.0 Hz, 1H), 3.03 (d, $J = 16.5$ Hz, 1H), 3.17 (d, $J = 17$ Hz, 1H), 3.68 (dd, $J = 9.0$, 5.5 Hz, 1H), 7.86 (s, 1H); HRMS (ESI): calculated for $C_{32}H_{52}O_9Na$ [M + Na]$^+$ 603.3509, found 603.3507.

Further elution provided spiroisomer **23** (9 mg, 55% for 3 steps) as a colourless oil: $[\alpha]_D^{17}$ −14.4 (*c* 0.30, CH_2Cl_2); ν_{max} (thin film) 2928, 1733, 1368, 1151 cm^{-1}; δ_H (400 MHz) 0.87 (t, $J = 8.0$ Hz, 3H), 1.25 (br s, 14H), 1.42 (s, 9H), 1.48 (s, 9H), 1.50 (s, 9H), 2.06 (t, $J = 8.0$ Hz, 2H), 2.24 (dd, $J = 13.2$, 7.2 Hz, 1H), 2.63 (t, $J = 12.8$ Hz, 1H), 2.87 (s, 2H), 3.53 (dd, $J = 12.4$, 6.8 Hz, 1H), 7.88 (s, 1H); δ_C (100 MHz) 14.1, 21.1, 22.7, 27.7, 27.9, 28.0, 28.0, 29.3, 29.3, 29.5, 31.8, 38.1, 39.5, 50.1, 81.1, 82.2, 82.3, 87.2, 108.4, 118.0, 168.0, 168.4, 169.6, 172.3, 198.1; HRMS (ESI): calculated for $C_{32}H_{52}O_9Na$ [M + Na]$^+$ 603.3509, found 603.3508.

(−)-Trachyspic acid (*ent*-1)

A solution of the tri-*tert*-butylester **23** (10 mg, 0.0172 mmol) in dry CH_2Cl_2 (1.5 cm^3) was cooled to 0 °C and treated with trifluoroacetic acid (TFA, 250 μL). The solution was stirred at 0 °C for 1 h, warmed to rt and stirred for an additional 2 h. Toluene (2.0 cm^3) was added and the solvent was removed under reduced pressure to afford *ent*-**1** (7.0 mg, 99%) as a thin film: $[\alpha]_D^{21}$ −3.5 (*c* 0.213, MeOH); $[\alpha]_D^{23}$ −8.4 (*c* 0.350, CH_2Cl_2); ν_{max} (thin film) 3425, 2927, 2856, 1724, 1611, 1370, 1139 cm^{-1}; δ_H (400 MHz, d_6-DMSO) 0.84 (t, $J = 6.6$ Hz, 3H), 1.23 (br s, 12H), 1.39 (m, 2H), 2.02 (t, $J = 7.8$ Hz, 2H), 2.36 (m, 2H), 2.67 (d, $J = 16.8$ Hz, 1H), 2.85 (d, $J = 16.8$ Hz, 1H), 3.56 (dd, $J = 11.8$, 7.8 Hz, 1H), 8.45 (s, 1H); δ_C (100 MHz, d_6-DMSO) 14.0, 20.5, 22.1, 27.5, 28.6, 28.7, 28.9, 31.3, 37.6, 38.7, 48.4, 86.5, 108.1, 116.7, 170.1, 170.6, 171.3,

174.5, 198.2; HRMS (ESI): calculated for $C_{20}H_{28}O_9H$ $[M + H]^+$ 413.1812, found 413.1809.

(−)-Trachyspic acid trimethyl ester (−)-25

A solution of (−)-trachyspic acid (*ent*-**1**) (7.0 mg, 15.8 μmol) in MeOH (1.0 cm^3) and Et_2O (250 μL) was cooled to 0 °C and treated with excess CH_2N_2. The solvent was removed under reduced pressure at 0 °C and the crude product was purified by flash chromatography with 20% EtOAc–petrol as eluent to give trimethyl ester (−)-**25** (3.0 mg, 42%) as a thin film: $[a]_D^{23}$ −20.4 (*c* 0.13, CH_2Cl_2); ν_{max} 2926, 2854, 1742, 1612, 1367, 1135 cm^{-1}; δ_H (400 MHz, d_6-DMSO) 0.84 (t, $J = 6.8$ Hz, 3H), 1.23 (br s, 12H), 1.38 (m, 2H), 2.02 (t, $J = 7.6$ Hz, 2H), 2.39 (dd, $J = 13.2$, 12.7 Hz, 1H), 2.47 (dd, $J = 13.2$, 7.4 Hz, 1H), 2.87 (d, $J = 16.8$ Hz, 1H), 2.93 (d, $J = 16.8$ Hz, 1H), 3.55 (s, 3H), 3.64 (s, 3H), 3.70 (s, 3H), 3.80 (dd, $J = 12.7$, 7.4 Hz, 1H), 8.47 (s, 1H); δ_C (100 MHz, d_6-DMSO) 14.0, 20.5, 22.2, 27.5, 28.7, 28.8, 29.0, 31.4, 37.3, 38.5, 47.7, 51.9, 52.6, 53.0, 86.5, 107.7, 117.0, 169.1, 169.5, 169.8, 174.7, 197.7; HRMS (ESI): calculated for $C_{23}H_{34}O_9Na$ $[M + Na]^+$ 477.2101, found 477.2093.

Heparanase inhibition assays

Reactions were set up in a volume of 100 μL containing 40 mM acetate buffer (pH 5.0), bovine serum albumin (BSA, 0.1 mg cm^{-3}), heparanase (90 ng), 5 μM [^{3}H]-HS and various concentrations of the trachyspic acid enantiomers. Initially, all components except the [^{3}H]-HS were allowed to equilibrate for 10 min at 22 °C. The assays were then initiated by adding [^{3}H]-HS and immediately 20 μL was taken, quenched with 80 μL of 10 mM phosphate (pH 7.0) and the 100 μL transferred to a Microcon YM-10 concentrator which was centrifuged at approximately 14 000 *g* for 5 min. The solution that passed through the membrane (filtrate) was retained. This sample was considered the time = 0 sample. The assays (now 80 μL in volume) were allowed to react at 37 °C for 4 h and then the filtration step was repeated for three aliquots of 20 μL from each assay. The time = 0 filtrate and the three time = 4 h filtrate samples were counted for ^{3}H using Optiphase HiSafe 2 scintillant (Perkin Elmer) in a Microbeta 1450 counter (Wallac).

The difference between the time = 0 and the averaged time = 4 h samples gave the amount of heparanase activity. Relative inhibition assays were run with a heparanase standard assay which was identical in composition except no inhibitor was present, and the amount of heparanase inhibition in the other assays was determined by comparison with this standard. IC_{50} values were determined for each compound.

Acknowledgements

This work was funded by an Australian Research Council Discovery Grant (DP0556064). We also thank Associate Professor Jonathan White for the X-ray crystallographic analysis and Dr Hideyuki Shiozawa (Sankyo Co., Ltd.) for copies of the NMR spectra of natural (+)-trachyspic acid (**1**) and trachyspic acid trimethyl ester (**25**).

Notes and references

1 For a recent review see: N. Ilan, E. Elkin and I. Vlodavsky, *Int. J. Biochem. Cell Biol.*, 2006, **38**, 2018.
2 For recent reviews see: E. Hammond, I. Bytheway and V. Ferro, in *New Developments in Therapeutic Glycomics*, ed. M. Delehedde and H. Lortat-Jacob, Research Signpost, Trivandrum, India, 2006, p. 251; E. A. McKenzie, *Br. J. Pharmacol.*, 2007, **151**, 1; H. Miao, H. Liu, E. Navarro, P. Kussie and Z. Zhu, *Curr. Med. Chem.*, 2006, **13**, 2101.
3 H. Shiozawa, M. Takahashi, T. Takatsu, T. Kinishita, K. Tanzawa, T. Hosoya, K. Furuya, S. Takahashi, K. Furihata and H. Seto, *J. Antibiot.*, 1995, **48**, 357.
4 K. Hirai, H. Ooi, T. Esumi, Y. Iwabuchi and S. Hatakeyama, *Org. Lett.*, 2003, **5**, 857.
5 H. Itazaki, K. Nagashima, Y. Kawamura, K. Matsumoto, H. Nakai and Y. Terui, *J. Antibiot.*, 1992, **45**, 38.
6 S. B. Singh, D. L. Zink, G. A. Doss, J. D. Polishook, C. Ruby, E. Register, T. M. Kelly, C. Bonfiglio, J. M. Williamson and R. Kelly, *Org. Lett.*, 2004, **6**, 337.
7 S. C. Zammit, J. M. White and M. A. Rizzacasa, *Org. Biomol. Chem.*, 2005, **3**, 2073.
8 R. Di Florio and M. A. Rizzacasa, *J. Org. Chem.*, 1998, **63**, 8595.
9 R. K. Mann, J. G. Parsons and M. A. Rizzacasa, *J. Chem. Soc., Perkin Trans. 1*, 1998, 1283.
10 A. N. Cuzzupe, R. Di Florio and M. A. Rizzacasa, *J. Org. Chem.*, 2002, **67**, 4392.
11 A. N. Cuzzupe, R. Di Florio, J. M. White and M. A. Rizzacasa, *Org. Biomol. Chem.*, 2003, **1**, 3572.
12 J. O. Bunte, A. N. Cuzzupe, A. M. Daly and M. A. Rizzacasa, *Angew. Chem., Int. Ed.*, 2006, **45**, 6376.
13 R. M. Giuliano and F. J. Villani, Jr., *J. Org. Chem.*, 1995, **60**, 202; T. Suami, K. I. Tadano, Y. Iimura and H. Yokoo, *J. Carbohydr. Chem.*, 1986, **5**, 1.
14 M. Zhao, J. Li, E. Mano, Z. Song, D. M. Tschaen, E. J. J. Grabowski and P. J. J. Reider, *J. Org. Chem.*, 1999, **64**, 2564.
15 L. J. Mathias, *Synthesis*, 1979, 561.
16 B. E. Rossiter and N. M. Swingle, *Chem. Rev.*, 1992, **92**, 771; C. Sahlberg, *Tetrahedron Lett.*, 1992, **33**, 679.
17 C. L. Kingsbury, K. S. Sharp and R. A. J. Smith, *Tetrahedron*, 1999, **55**, 14693.
18 X-Ray analysis established the relative configuration. There were two molecules in the asymmetric unit. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 268561. See http://www.rsc.org/suppdata/ob/b5/b504258e for crystallographic data in CIF format.
19 R. Laschober and W. Stadlbauer, *Liebigs Ann. Chem.*, 1990, **11**, 1083.
20 P. G. McDougal, J. G. Rico, Y. Oh and B. D. Condon, *J. Org. Chem.*, 1986, **51**, 3388.
21 E. J. Corey and P. L. Fuchs, *Tetrahedron Lett.*, 1972, **13**, 3769.
22 H. Shoji, D. Hedetaka, T. Satoru and S. Akira, *Tetrahedron Lett.*, 1983, **24**, 731.
23 W. F. Bailey and E. R. Punzalan, *J. Org. Chem.*, 1990, **55**, 5404.
24 S. F. Martin and J. A. Dodge, *Tetrahedron Lett.*, 1991, **32**, 3017.
25 T. Karoli, L. Liu, J. K. Fairweather, E. Hammond, C. P. Li, S. Cochran, K. Bergefall, E. Trybala, R. S. Addison and V. Ferro, *J. Med. Chem.*, 2005, **48**, 8229.

