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Short communication

Comparative action of carbocyclic thromboxane A_2 stereoisomers on platelets

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Abstract – Stereospecific requirements for the interaction of the thromboxane A_2 carbocyclic mimetic CTA_2 **1** with the human platelet PGH_2/TXA_2 receptor have been explored. The two pairs of *trans*-**1**,**2** and *cis*-**3**,**4** side chain diastereoisomers were synthesised and evaluated for agonist and antagonist activity in human platelet rich plasma. Interestingly, the natural and unnatural *trans* diastereoisomers, both possessed potent aggregatory activity and equipotently inhibited platelet responses to subsequent addition of agonists, whereas, the respective unnatural *cis* isomers proved only weakly active or inert. © 2000 Éditions scientifiques et médicales Elsevier SAS

carbocyclic / platelets / stereoisomers / thromboxane A_2

1. Introduction

We [1, 2] and others [3–5] have earlier reported the synthesis of analogues of thromboxane A_2 (TXA_2), including the carbocyclic analogue CTA_2 **1** *table I*, in which the unstable cyclic ether is replaced by carbon groupings. Although the synthesis and pharmacology of natural TXA_2 [6, 7], CTA_2 **1** [8] and numerous other stable mimetics, such as 9,11-methanoepoxy PGH_2 (U-46619) and 9,11-azo PGH_2 [9–12], have been extensively described, there is, as far as we are aware, no published comparative biological data on the four possible α-chain and ω-chain (*trans*/*cis*) diastereoisomers for any of these structures. Here, we report the synthesis and a comparative study of the PGH_2/TXA_2 receptor agonist and antagonist properties of the four side chain stereoisomers **1**–**4** of CTA_2 using human platelet rich plasma (PRP). Data obtained in the same test system for pinane TXA_2 (PTA_2) **5** and its 15-epimer **6** are included for reference and comparison.

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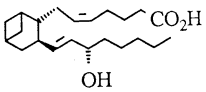
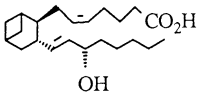
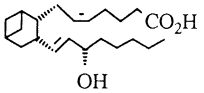
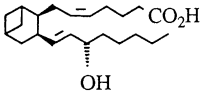
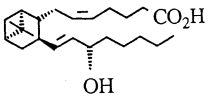
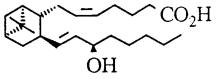
1.1. Chemistry

CTA_2 **1**, and the ent-15-epi diastereoisomer **2** (*table I*) were synthesised, using a modification of the published procedure [4], via conjugate addition of the cuprate **10** to the bicycloheptene aldehyde **9** [2] (*figure 1*). We found that the latter which had earlier been synthesised by Nicolaou et al. from bicyclo {2.1.1} hexanone and from cyclohexane-1,4-dione [4], could be made in 70% yield by pyrrolidinium acetate-catalysed cyclisation of the symmetrical dialdehyde **8d**.

The dialdehyde **8d** was synthesised from the ditosylate **8b** by chain extension with sodium cyanide in DMSO to the dinitrile **8c** and then reaction with di-isobutylaluminium hydride. The ditosylate was prepared by a modification of the literature route from the anhydride **7** [13] using bis (2-methoxyethoxy) aluminium hydride for reduction to the diol **8a** followed by tosylation at low temperature, which gave improved yields.

Conjugate addition of the optically active cuprate **10** to the bicycloheptene aldehyde **9** using the method of Corey and Beames [14] gave a diastereoisomeric mixture of the two *cis* kinetic products **11a** and **11b** and the *trans* thermodynamic products **11c** and **11d**. Treatment with an organic base 1,5-diazabicyclo[4.3.0]non-5-ene (DBN)

Table I. The effect of thromboxane A_2 analogues on aggregation of human platelets [a].

Compd	Structure	References	Aggregatory action (μM)	Inhibition of aggregation (Ic_{50} μM) induced by:		
				Collagen	Arachidonic acid	U-46619 [b]
1		[2]	3–100 [c] (5)	27 (5)	26 (5)	17.5, 4.6
2		[2]	3–100 [c] (5)	20 (5)	6.6 (5)	19.0, 4.5
3[d]			>100 (6)	>100 (6)	70 (5)	>100, 32.5
4[d]			>100 (6)	>100 (6)	>100 (4)	
5		[1]	>130 (7)	>130 (7)	20 (5)	46.0, 24.5
6		[1]	>130 (3)	120–130 (3)		

[a] All the results are means of three or more experiments (number of experiments in parenthesis) on PRP samples from different donors except U-46619 where results of two duplicate experiments are both quoted.
[b] (15S)-Hydroxy-llα,9α-(epoxymethano)prosta-5Z,13E dienoic acid.
[c] Weak reversible activity.
[d] Relative configurations of *cis* isomers **3** and **4** not established; **3** is the more polar isomer on TLC.

isomerised the *cis* isomers **11a** and **11b** to afford an all *trans* mixture **11c** and **11d**, which was subjected to a one methylene unit chain extension by means of a Wittig condensation with the ylid generated from (methoxymethyl)triphenylphosphonium chloride using lithium diisopropylamide. Cleavage of the resulting vinyl ethers **12c** and **12d** (obtained as a *cis*/*trans* mixture about the double bond) using mercury(II) acetate and potassium iodide, then gave the aldehydes **13c** and **13d**, which underwent Wittig reaction with the ylid generated from (4-carboxybutyl)triphenylphosphonium bromide and potassium *t*-butoxide in tetrahydrofuran to afford a mixture of the acids **14c** and **14d**. Desilylation gave a 5:4 mixture of CTA_2 **1** and its diastereoisomer **2**, which were separated by HPLC. Our elaboration of the aldehyde **9** was essentially similar to that described by Nicolaou's group [4], the main difference being their preparation of the methyl ester of the ω-chain, which required hydrolysis to liberate CTA_2.

The structures of the isomers **1** and **2** were assigned on the relative order of their chromatographic mobilities, the method used by the Nicolaou group [4], which is in accordance with the general finding with prostanoid analogues that the more polar of an isomer pair has the same configuration as the corresponding naturally occurring compound.

We found that a mixture of all the four isomers **12a**–**d** could be obtained, if base treatment of the mixture **11a**–**d** was omitted and the subsequent Wittig reaction carried out without delay to avoid isomerisa-

tion of the *cis* isomers. Elaboration, as described above, gave a mixture containing the four diastereoisomers of CTA_2 **1**, **2**, **3** and **4**, which were separated by HPLC.

Assignments of the relative configurations of the earlier unreported two *cis* isomers **3** and **4** could not be made with the data available.

2. Results and discussion

The biological results are shown in *table I*.

CTA_2 **1** and its ent-15-epi isomer **2** demonstrated significant, concentration-related, reversible (1–2 min) aggregatory activity on human PRP at final concentrations in the range 3.0–100.0 μM (*figure 2*). The effect was of a similar magnitude for both the compounds at equivalent concentrations, donor dependent, and maximal at 30 or 100 μM (15–20% increase in light transmission). In contrast, the two *cis* diastereoisomers **3** and **4** possessed no intrinsic aggregatory activity at concentrations up to 100 μM.

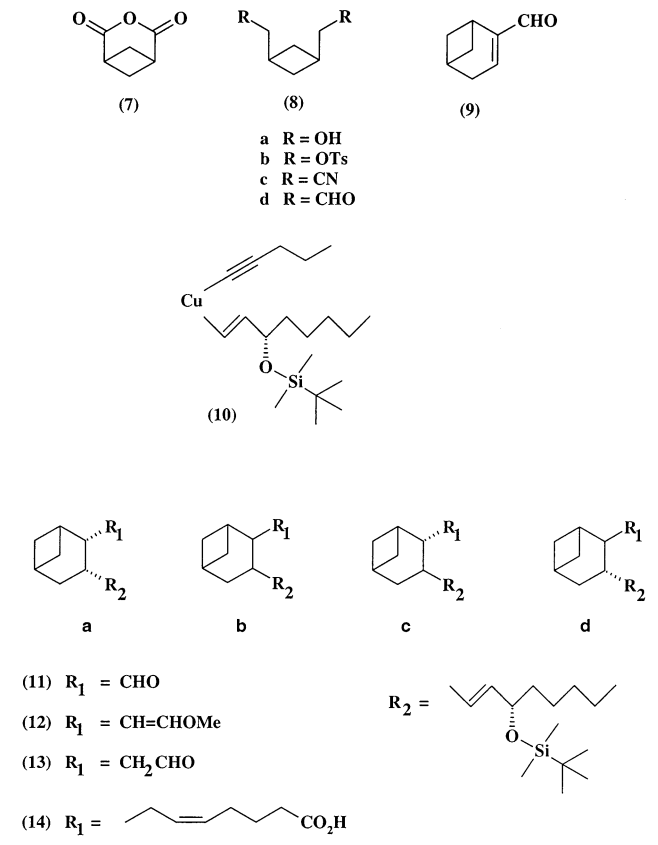


Figure 1. Structure of intermediates.

CTA_2 **1** and its ent-15-epi isomer **2** both also inhibited aggregation of human PRP induced by all three agonists under test. They were equipotent against collagen-induced aggregation (IC_{50} values: **1**, 27 ± 9, **2**, 20 ± 7 μM (mean ± S.E.M.)) and U-46619-induced aggregation (duplicate experiments only), but the ent-15-epi isomer **2** appeared substantially more potent (IC_{50} 6.6 ± 1.2 μM) than CTA_2 **1** itself (IC_{50} 26 μM) against arachidonic acid-induced aggregation. *cis* isomer **3** showed marginal activity against arachidonic acid and U-46619-induced aggregation, but was inactive against collagen-induced aggregation. *cis* isomer **4** was inactive against collagen and arachidonic acid-induced aggregation at concentrations up to 100 μM.

In the same PRP preparations, PTA_2 **5** and its 15-epimer, **6** [1] were devoid of aggregatory activity at the concentrations examined. PTA_2, however, exhibited moderate inhibitory activity against arachidonic acid and U-46619-induced platelet responses.

The observation here of moderate, concentration-dependent, reversible aggregatory activity in vitro at plasma CTA_2 concentrations in the range 3.0–100.0 μM is in good agreement with an earlier report describing weak, reversible responses induced in PRP by CTA_2 at 20 μM [8]. Armstrong et al. [15] have noted shape change and reversible aggregation at 0.5–10.0 μM CTA_2 in diluted PRP and 50–250 μM in plasma free platelet suspensions. Equally, CTA_2 **1** also demonstrated dose-dependent activity as an inhibitor of platelet responses to subsequent addition of either arachidonic acid, collagen or U-46619 as reported earlier [8, 15]. Burke et al. [8] and Lefer et al. [16] also saw this effect at a concentration of 1–5 μM in PRP versus 9,11-azo PGH_2. Since the initial platelet shape change and reversible aggregatory response induced by CTA_2 can be blocked by the selective PGH_2/TXA_2 receptor antagonist EP045 [15] and CTA_2 actively displaces ^{3}H-15(S)-9,11-epoxymethano PGH_2 from human platelet binding sites [17], the data are consistent with the view that CTA_2 is a potent partial agonist at the human platelet PGH_2/TXA_2 receptor.

It is of particular interest that the ent-15-epi isomer of CTA_2 **2** possesses a similar profile of activity and level of potency on the platelet to CTA_2 itself **1**. By analogy, it is possible that the other isomer pairs with the same skeletal relationships may have similar relative levels of potency and that the so far unknown ent-15-epi TXA_2 might have a level of potency of the same order as that of the natural TXA_2. It is also possible that the same principle may apply in other

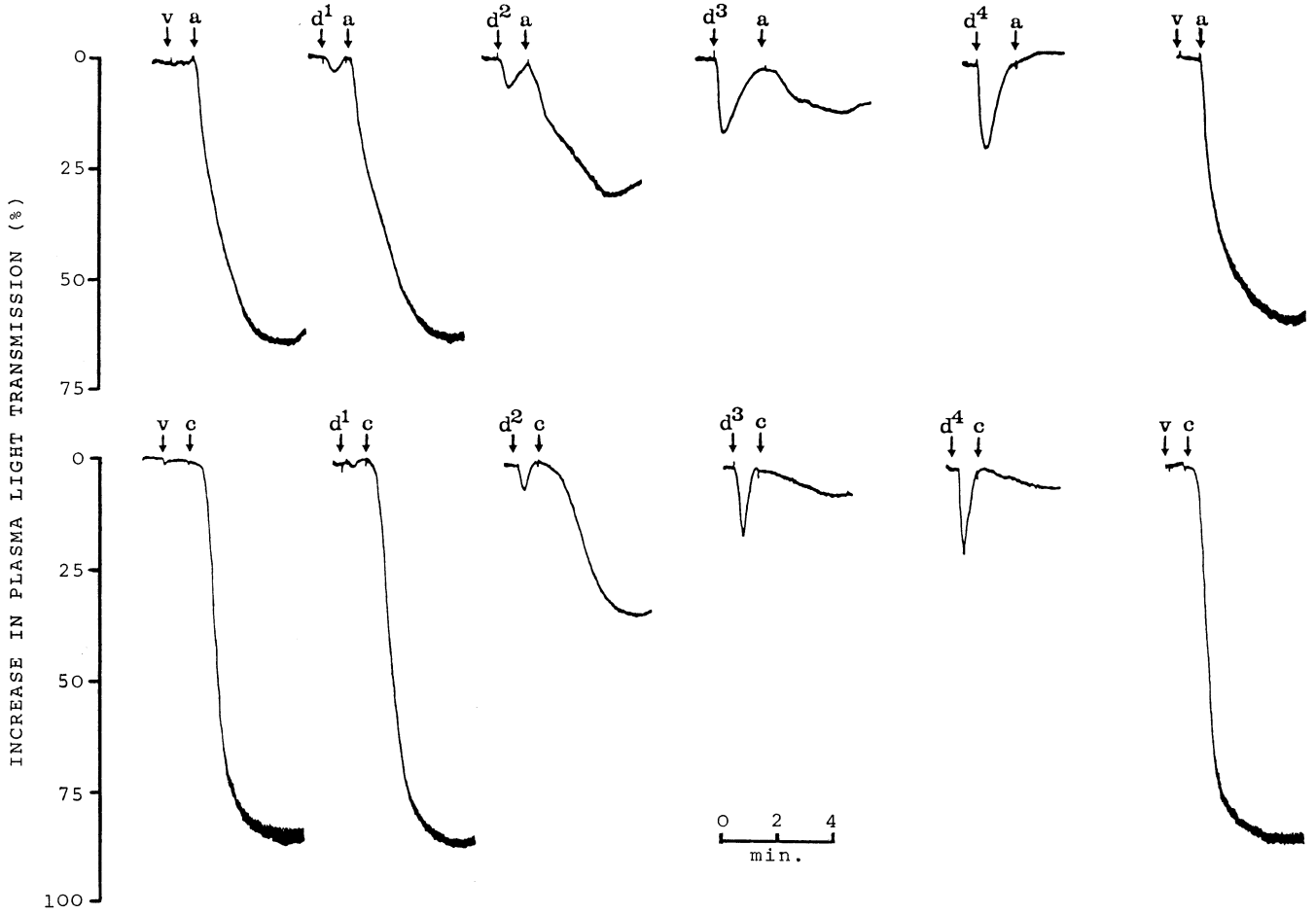


Figure 2. Effect of CTA_2 **1** and its ent-15-epi-stereoisomer **2** on aggregation in human platelet rich plasma in vitro. Sequence 1 (upper panel) shows the effect of CTA_2 **1** on 1.0 mM arachadonic acid (a)-induced aggregation. Sequence 2 (lower panel) shows the effects of the CTA_2 stereoisomer **2** on 2.0 μg mL^{-1} collagen (c)-induced aggregation. Test compounds were incubated with PRP for a period of 1–2 min (depending on the duration of the reversible response) before the addition of agonist, d^1–d^4 representing 3.0, 10.0, 30.0 and 100.0 μM final concentrations in both cases. Vehicle controls (v) were carried out prior to and following the drug determinations. The traces shown are representative of the aggregatory profile observed for both compounds against either platelet agonist on each of five separate occasions.

prostanoid series and it is of interest that evidence has been presented of high levels of biological activity of the ent-15-epi- stereoisomers of PGE_1 and PGA_1 [18].

In sharp contrast to the *trans* compounds, the diastereoisomers **3** and **4** in which the α and ω side chains are in the *cis* relationship exhibited no agonist activity at the concentrations tested, and only weak, marginal inhibitory activity in one case **3**.

A correct mechanistic interpretation of the inhibitory effects of CTA_2 **1** and its ent-15-epi isomer **2** in PRP on subsequent addition of a second agonist is complex and requires caution. The initial reversible platelet aggregatory response obtained in the presence of CTA_2 may induce a measure of receptor desensitisation or tachyphylaxis in the platelet. In addition, since CTA_2 markedly increases intracellular cyclic 3′,5′-AMP levels ($>$2-fold at 1.25 μM) in washed human platelet preparations [15], this factor may contribute to both the rapidly reversible nature of the initial aggregatory response to CTA_2, and also, by functional antagonism, to the observed attenuation of the response following addition of a second agonist.

In addition to effects on platelets, it has been reported by Lefer et al. [16] that compound **1** has

potent low mM vasoconstrictor effects. It would thus be of interest in further studies to investigate the comparative activities of the isomers **1**–**4** on vascular smooth muscle.

Finally, we also found PTA_2 to be less potent as an inhibitor of arachidonic acid and U-46619-induced aggregation in human PRP than reported earlier [8]. This compound was reported by Burke et al. [8] to completely inhibit arachidonic acid induced aggregation at 20 μM. Nonetheless, as PTA_2 exhibited no agonist properties of the type displayed by CTA_2 **1** and its ent-15-epi isomer **2** in our experiments, the data suggest that it possesses weaker, but pure antagonist activity at the platelet PGH_2/TXA_2 receptor. However, caution is again required, since PTA_2 is reported to cause shape change in plasma free platelet suspensions [15] and has shown partial agonist properties on certain thromboxane sensitive smooth muscle preparations [19].

3. Experimental protocols

3.1. Human platelet aggregation

Platelet assays were performed by standard turbidometric methods using citrated human PRP standardised to 2.5×10^8 cells mL^{-1} in a 2-channel platelet aggregometer (Medcontrol Ltd) connected to a pen recorder (Bryan's Southern Instruments Ltd). PRP (0.6 mL) was equilibrated to 37°C, and stirred at 1000 rpm for 2 min. A maximum of 2.4 μL of the test compound in absolute ethanol was added to the pre-incubated PRP, which was further incubated for 1–2 min before inducing aggregation, by addition of either arachidonic acid (Sigma) (0.3–1.0 mM), collagen (Hormon Chemie) (2 μg mL^{-1}), or U46619[1] (3.0 μM) [9]. The aggregation response was recorded by an increase in light transmission at a wavelength of 660 nm.

4. Chemistry

^1H and ^{13}C NMR spectra were measured in $CdCl_3$ solution, unless otherwise noted, on Varian A60D, EM360 or CIFT 20 spectrometers. IR spectra were measured on thin films (liquids) and KBr discs (solids) on a Unicam SP1000 spectrophotometer. UV spectra were measured in ethanol solution on a Carey 17 spectrophotometer. TLC and column chromatographs were carried out using silica gel. LPLC refers to column chromatography on plate grade silica gel with a slight pressure (ca. 5 psi) applied.

4.1. cis-1,3-bis(Hydroxymethyl)cyclobutane **8a**

To a stirred solution of bis(2-methoxyethoxy) aluminium hydride in benzene (70% solution, 0.2 mol, 56.2 mL) under nitrogen, a solution of the anhydride of cyclobutane-*cis*-1,3-dicarboxylic acid **7** [13] (6.26 g, 0.05 mol) in benzene (50 mL) was added dropwise. The mixture was stirred at reflux for 2 h, then cooled and treated dropwise with hydrochloric acid (aq 10%) to pH 2. The mixture was saturated with sodium chloride and extracted with ether continuously for 18 h. The ethereal extract was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil which was distilled to give the diol **8a** (5.4 g, 83%) as a colourless oil b.p. 82–87°C at 0.5 mmHg (lit. [13] 107–108°C at 1.3 mmHg); IR ν_{max} 3600–3100 cm^{-1} (OH).

4.2. Ditosylate of cis-1,3-bis(Hydroxymethyl)-cyclobutane **8b**

The diol **8a** (4 g, 34.5 mmol) was added to a solution of toluene-*p*-sulphonyl chloride (18.7 g, 0.1 mol) in dry pyridine (50 mL) at −5°C. The mixture was left at 2°C for 18 h, then poured on to crushed ice and extracted with diethyl ether (2 × 100 mL). The extracts were dried (Na_2SO_4 and concentrated under reduced pressure to give a white solid, which was recrystallised from ether/*n*-hexane to give the ditosylate **8b** (13.8 g, 94%) m.p. 77–78°C (lit. [13] 76.5–77°C). Anal. $C_{20}H_{24}O_6S_2$.

4.3. cis-1,3-bis(Cyanomethyl)cyclobutane **8c**

Sodium cyanide (7.6 g, 157 mmol) was added to a stirred solution of the ditosylate **8b** (13.0 g, 30.6 mmol) in dry dimethylsulphoxide (150 mL). The reaction mixture was stirred for 18 h at room temperature, diluted with saturated sodium chloride solution (200 mL) and extracted with diethyl ether (3 × 200 mL). The combined extracts were washed with water (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow solid, which was recrystallised from *n*-hexane to give the *dinitrile* **8c** (2.75 g, 67%) as an off white crystalline solid, m.p. 52–54°C. Anal. $C_8H_{10}N_2$. IR ν_{max}

[1] (15S)-Hydroxy-11α,9α(epoxymethano)prosta-5,13-dienoic acid.

2985 (cyclobutane) and 2240 cm^{-1} (C≡N); H-NMR δ_H 80 MHz) 2.2–2.7 (8H, m), 1.2–1.9 (2H, bm); C118.06 (s, C≡N), 32.70 and 23.45 (t, CH_2), 27.35 (d, CH).

4.4. *cis-1,3-bis(Formylmethyl)cyclobutane* ***8d***

To a stirred solution of the dinitrile **8c** (1.34 g, 10 mmol) in dry diethyl ether (60 mL) under nitrogen at 0°C was added a solution of di-isobutylaluminium hydride (5.7 g, 40 mmol) in dry benzene (25 mL). The mixture was stirred for 1.5 h, added dropwise (exothermic) to acetic acid (2 M, 100 mL), and then extracted with diethyl ether (3×200 mL). The extracts were washed in turn with water (2×100 mL), and saturated aqueous sodium hydrogen carbonate then dried (Na_2SO_4) and concentrated under reduced pressure to give the *dialdehyde* **8d** (1.17 g, 84%) as a pale yellow oil (MS M+, 140. $C_8H_{12}O_2$; R_F (diethyl ether) 0.47; IR ν_{max} 2620 and 1725 cm^{-1} (CHO); H-NMR δ_H (80 MHz) 9.55 (2H, t, $J = 2$ Hz, CHO), 2.55 (8H, m), 1.45 (2H, m)).

4.5. *2-Formylbicyclo[3.1.1]hept-2-ene* ***9***

To a solution of the dialdehyde **8d** (0.84 g, 6 mmol) in dry benzene (30 mL) was added pyrolidine (2 drops) and glacial acetic acid (3 drops) and the resulting mixture was heated at reflux for 1.5 h under nitrogen. The cooled mixture was diluted with diethyl ether (50 mL) and washed successively with hydrochloric acid (2 M, 20 mL), aqueous sodium carbonate solution (2 M, 20 mL), water (40 mL) and dried (Na_2SO_4). The dried extract was concentrated under reduced pressure to give a light brown oil (0.66 g), which was purified by LPLC on a column of silica gel (50 g, 2.5×30 cm) using diethyl ether as eluant to give the aldehyde **9** (0.51 g, 70%) [4] as an oil (MS MS^+, 122. $C_8H_{10}O$; R_F (diethyl ether) 0.53; UV λ_{max} (EtOH) 241 nm ε 9400); IR ν_{max} 3010, 1680 (conj CHO), and 1622 cm^{-1} (conj C=C); H-NMR δ_H (80 MHz) 9.4 (1H, s, CHO), 6.65 (IH, m, C=C*H*), 3.15 (1H, m, C*H*) 2.65 (3H, m), 2.25 (2H, m), 1.20 (2H, m).

4.6. *3-[IE,3S,3-(t-Butyldimethylsilyloxy)oct-1-enyl]-2-formylbicyclo [3.1.1] heptane* ***11a–d***

To a stirred solution of IE,3S,3-(*t*-butyldimethylsilyloxy)-l-iodo-oct-l-ene [20] (2.24 g, 6.05 mmol) in dry diethyl ether (15 mL) under argon at −78°C was added a solution of *t*-butyllithium (2M, 6 mL, 12 mmol) in pentane and the mixture was stirred for 2 h. Hexamethylphosphorous triamide (2.36 mL, 12.3 mmol) was added to a stirred suspension of pentynylcopper [14] (0.83 g, 6.4 mmol) in dry diethyl ether (15 mL) under argon and the mixture was stirred until a clear solution formed (ca. 15 min). The solution of the copper complex was then added dropwise to the lithium salt reaction mixture, followed 0.5 h later by a solution of the aldehyde **9d** (0.7 g, 5.7 mmol) in dry diethyl ether (10 mL). The stirred reaction mixture was kept at −75°C for 2 h, and was then warmed to −4°C for 0.75 h, and added to aqueous ammonium sulphate (20% w/v, 200 mL) at 0°C. The aqueous phase was washed with diethyl ether ($2 \times$ 100 mL) and the combined organic material was treated with sulphuric acid (2%, v/v, 200 mL) and filtered through Celite[2]. The separated organic material was washed with aqueous sodium hydrogen carbonate solution (5% w/v, 200 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give a brown oil (2 g). The oil was purified using LPLC on a column of silica gel (70 g, 3.5×16.5 cm) using diethyl ether/*n*-hexane (5:95) as eluant to give a mixture the isomeric *aldehydes* **11a–d** (0.71 g, 34%) as an oil, R_F [diethyl ether/*n*-hexane (1:19)] 0.34; IR ν_{max} 2718, 1728, (CHO), 1255 [Si$(CH_3)_2$], and 770 cm^{-1} (C=C); H-NMR δ_H (80 MHz) 9.88 (0.5H, d, $J = 2.5$ Hz, *cis* CHO), 9.65 (0.5H, d, $J = 2.5$ Hz, *trans* CHO), 5.5 (2H, m C*H*=C*H*), 4.1 (1H, m, *H*COSi), 0.8–3.5 (30H, m), 0.1 [6H, s, Si$(CH_3)_2$].

4.7. *3-[IE,3S,3-(t-Butyldimethylsilyloxy)oct-1-enyl]-2-(2-methoxyvinyl) bicyclo[3.1.1]heptane* ***12a–d***

To a stirred solution of *n*-butyllithium (1.4 M, 3.5 mL, 4.84 mmol) in *n*-hexane under nitrogen at −78°C was added a solution of di-isopropylamine (1.0 mL, 7 mmol) in dry tetrahydrofuran (10 mL). After 0.5 h the solution was added dropwise to a stirred suspension of (methoxymethyl)triphenylphosphonium chloride (1.6 g, 4.7 mmol) in dry tetrahydrofuran (10 mL) at −75° C under nitrogen. The mixture was stirred for 0.5 h and then allowed to warm to 0°C during 0.5 h, and a solution of the aldehydes (**11a–d**) (0.6 g, 1.6 mmol) in dry tetrahydrofuran (10 mL) was added dropwise. The mixture was stirred for 2 h and then concentrated under reduced pressure at 40°C. The residue was dissolved in water and extracted with diethyl ether (2×50 mL). The

[2] Johns–Manville.

combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a dark brown oil (1.1 g). An analytical sample (0.1 g) was purified by LPLC on silica gel (16 g, 2.4 × 16 cm) using ether/*n*-hexane (1:19) as eluant to give the isomeric *vinyl ethers* **12a**–**d** (50 mg, 85%) as a colourless oil. MS 392, C_{24} $H_{44}O_2Si$; R_F (diethyl ether) 0.61; IR ν_{max} 1670, 1650 (C=C–OCH_3), 1260 [$Si(CH_3)_2$], and 980 cm^{-1} (C=C), H-NMR δ_H (60 MHz) 4.5–6.5 (4H, m, *H*C=C*H*) 3.8–4.2 (1H, m, C*H*OSi), 3.5, 3.45 (3H, 2 s, *cis* and *trans* OCH_3), 1.0–2.6 (18H, m), 0.85 (12H, C–CH_3), 0.03 [6H, s, $Si(CH_3)_2$].

4.8. 3-[IE,3S,3-(t-Butyldimethylsilyloxy)oct-1-enyl-2-(formylmethyl)bicyclo[3.1.1]heptane **13a–d**

A stirred solution of the vinyl ethers (**12a**–**d**) (1.0 g, 2.5 mmol) in a 1:10 mixture of water–THF (13 mL) was treated with mercury(II) acetate (2.5 g, 7.7 mmol) and after 15 min saturated aqueous potassium iodide (33 mL) was added. After 5 min the reaction mixture was extracted with diethyl ether (2 × 50 mL) and the combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil (0.7 g), which was purified by LPLC on a silica gel (100 g) column (3.5 × 18 cm) with diethyl ether/*n*-hexane (5:95) as eluant to give the mixture of isomeric *aldehydes* (**13a**–**d**) (0.37g, 38%) as a colourless oil R_F [diethyl ether/*n*-hexane (1:19)] 0.16; IR ν_{max} 2718 (CHO), 1728 (CHO), 1255 [$Si(CH_3)_2$] and 970 cm^{-1} (C=C); H-NMR δ_H (80 MHz) 9.7 (1H, t, $J = 1.5$ Hz, CHO), 5.45 (2H, m, CH=CH), 4.05 (1H, m, CHOSi), 1.0–2.75 (32H, m), 0.11, 0.1 [together 6H, 2S, $Si(CH_3)_2$].

4.9. 3-[1E,3S,3-(t-Butyldimethylsilyloxy)oct-1-enyl]-2(Z,6-carboxyhex-2-enyl)bicyclo[3.1.1]heptane **14a–d**

A stirred suspension of (4-carboxybutyl)triphenylphosphonium bromide (1.26 g, 2.84 mmol) in dried THF (20 mL) under argon at room temperature was treated with a solution of potassium *t*-butoxide (0.81 g 7.1 mmol) in dried THF (20 mL). The mixture was stirred for 20 min and then treated with a solution of the aldehydes (**13a**–**d**) (0.27 g, 0.71 mmol) in dried THF (5 mL). After 2.5 h water (10 mL) was added and the reaction mixture stirred for a further 0.5 h. The reaction mixture was diluted with water (100 mL) then washed with diethyl ether (50 mL) and the ether phase was washed with saturated aqueous sodium hydrogen carbonate (50 mL). The combined aqueous material was acidified to pH 2 using diluted hydrochloric acid, and extracted with diethyl ether (2 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow oil (1.5 g), which was purified by LPLC on a silica gel (70 g) column (3.4 × 16.5 cm) with diethyl ether/*n*-hexane (1:19) as eluant to give the isomeric mixture of *acids* (**14a**–**d**) (0.25 g, 76%) as an oil MS M + 462 $C_{20}H_{50}O_3$ Si R_F (diethyl ether) 0.53; IR ν_{max} 1710 (CO_2H), 1260 [$Si(CH_3)_2$], and 970 cm^{-1} (C=C); H-NMR δ_H (80 MHz) 7.0 (1H, br, OH, exchangeable), 5.4 (4H, m, 2 × CH=CH), 4.1 (1H, m, CHOSi), 0.7–2.5 (26H, m), 0.9 [12H, bs, $SiC(CH_3)_3$ and CH_2 CH_3), 0.05 [6H, s, $Si(CH_3)_2$].

4.10. 2-(2Z-6-Carboxyhex-2-enyl)-3-(IE,3S,3-hydroxyoct-1-enyl)bicyclo[3.1.1]heptane-trans **1**,**2** and cis isomers **3**,**4**

A solution of the acids **14a**–**d** (0.1 g, 0.22 mmol) in a mixture of glacial acetic acid, water, and THF (6:4:1; 11 mL) was stirred at 40–45°C for 3 h. The reaction mixture was cooled and diluted with water (10 mL) and extracted with diethyl ether (3 × 20 mL). The combined extracts were washed with water (20 mL) several times until the pH of the washings was 4. The ethereal solution was dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow oil (0.8 g). (MS M + 348; $C_{22}H_{36}O_3$). IR ν_{max} 2600–3400, 1710 (CO_2H), and 970 cm^{-1} (C=C); H-NMR δ_H (80 MHz) 6.2 (2H, b, OH, chemical shift dependent on concentration), 5.1–5.7 (4H, m, 2 × r C*H*=C*H*), 4.10 (1H, m, C*H*OH), 0.6–2.6 (29H, m). This mixture was separated by HPLC using *n*-hexane/ethyl acetate/acetic acid (700:100:1) to give in order of elution: *trans* isomer **2**, [4] mixture of *cis isomers* **3** and **4** and *trans* isomer **1** (CTA_2) [4]. The two components of the *cis* isomer mixture were then separated by HPLC using *n*-hexane–dichloromethane–acetic acid (700:180:1) as eluant.

4.11. 2E,3-[1E,3S,3-(t-Butyldimethysilyloxy)oct-1-enyl]-2-formylbicyclo[3.1.1]heptane **11c**,**d**

Following the same procedure and scale for the preparation of **11a**–**d** except that before drying with Na_2SO_4 the extracts were treated with DBN (1 mL, 8.1 mmol) and the mixture stirred at room temperature for 18 h. The mixture was then dried (Na_2SO_4) and concentrated under reduced pressure to give a brown oil (1.2 g), which was purified by LPLC on a silica gel (16 g) column (2.4 × 15 cm) using diethyl ether: *n*-hexane

(1:19) as eluant to give the mixture of *trans aldehydes* **11c** and **11d** (0.21 g, 20%) as an oil. (MS M + 393; $C_{24}H_{44}O_2$ Si M 393); R_F [diethyl ether/*n*-hexane (1:19)] 0.34. IR ν_{max} 2620 (CHO), 1725 (CHO), 970 cm^{-1} (C=C), H-NMR δ_H (80 MHz) 9.65 (1H, bs, CHO), 5.5 (2H, m, CH = CH), 4.1 (1H, m, CHOSi) 0.8–3.5 (30H, m), 0.1 [6H, s, $Si(CH_3)_2$].

4.12. 2E,3-[1E,3S,3-(t-Butyldimethylsilyloxy)oct-1-enyl]-2-(2-methoxyvinyl)bicyclo[3.1.1]heptane ***12c,d***

Following the procedure for the preparation of **12a**–**d** the *trans* aldehydes **11c** and **11d** (0.2 g, 0.53 mmol) in dry THF (6.5 mL) were treated, as earlier, with the ylide prepared by treating lithium di-isopropylamide [n-butyl lithium (1.2 M in hexane, 1.34 mL, 1.6 mmol) and di-isopropylamine (0.33 mL, 2.3 mmol) in dry THF (6.5 mL)] with (methoxymethyl)triphenylphosphonium chloride (0.53 g, 1.5 mmol) in dry THF (6.5 mL) to give the *vinyl ethers* (**12c**,**d**) (0.13 g, 60%) with the same spectral properties (IR and NMR) as described for **12a**–**d**. R_F (diethyl ether) 0.60.

4.13. 2E,3-[1E,3S,3-t-Butyldimethylsilyloxy)oct-1-enyl]2(formylmethyl)bicyclo[3.1.1]heptane ***13c,d***

A stirred solution of the vinyl ethers **12c**,**d**, (0.11 g, 0.28 mmol) in a 1:10 mixture of water–THF (1.5 mL) was treated with mercury(II) acetate (0.27 g, 7.9 mmol) and after 15 min saturated aqueous potassium iodide (3.6 mL) was added. After 5 min the reaction mixture was extracted with diethyl ether (2 × 20 mL) and the combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil (0.14 g), which was purified by LPLC on a silica gel (16 g) column (2.4 × 15 cm) with diethyl ether/*n*-hexane (1:19) as eluant to give the *aldehyde* **13c**,**d** as an oil (0.07 g, 66%) with the same spectral properties (IR) as described for **13a**–**d** R_F [hexane:diethyl ether (95:5)] 0.17.

4.14. 2E,3-[1E,3S,3-(t-Butyldimethylsilyloxy)oct-1-enyl-2(2Z,6,carboxyhex-2-enyl)bicyclo[3.1.1]heptane ***14c,d***

Following the procedure for the preparation of **14a**–**d** the α-chain phosphonium ylide was generated by treating (4-carboxybutyl)triphenylphosphonium bromide (0.28 g, 0.63 mmol) with potassium *t*-butoxide (0.18 g, 1.58 mmol) in THF (7 mL). The ylide was then treated with the aldehyde **13c**,**d** (0.06 g, 0.16 mmol) and after work up, as described earlier, gave a dark brown oil (0.12 g), which was purified by LPLC on a silica gel (12 g) column (2.4 × 11 cm) using diethyl ether as eluant to give the *trans acids* **14c**,**d** as an oil (0.06 g, 82%) with the same spectral properties as **14a-d** (NMR). R_F (diethyl ether) 0.53.

4.15. 2E,2-(2Z,6-Carboxyhex-2-enyl)-3-(1E,3S,3-hydroxyoct-l-enyl) bicyclo[3.1.1]heptane-trans isomers ***1***, ***2***.

A solution of the *trans* acids **14c** and **14d** (0.04 g, 0.09 mmol) in a mixture of glacial acetic acid, water and THF (6:4:1; 11 mL) was stirred at 40–45°C for 3.5 h. The reaction mixture was cooled and diluted with water (5 mL) and extracted with diethyl ether 3 × 10 mL). The combined extracts were washed with water (10 mL) several times until the pH of the washings was 4. The ethereal solution was dried ($MgSO_4$) and concentrated under reduced pressure to give an oil, which was separated by HPLC into the two *trans* diastereoisomeric acids-**1** (CTA_2) [4] 10.5 mg, 70% (the more polar isomer) and the isomer **2** [4] (8.5 mg, 57%), using *n*-hexane–ethyl acetate–acetic acid (700:100:1) as eluant [K for **1** is 3.1 and for **2** is 4.8].

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