15-Deoxy-16-(*m*-tolyl)-17,18,19,20-tetranorisocarbacyclin: a simple TIC derivative with potent anti-apoptotic activity for neuronal cells

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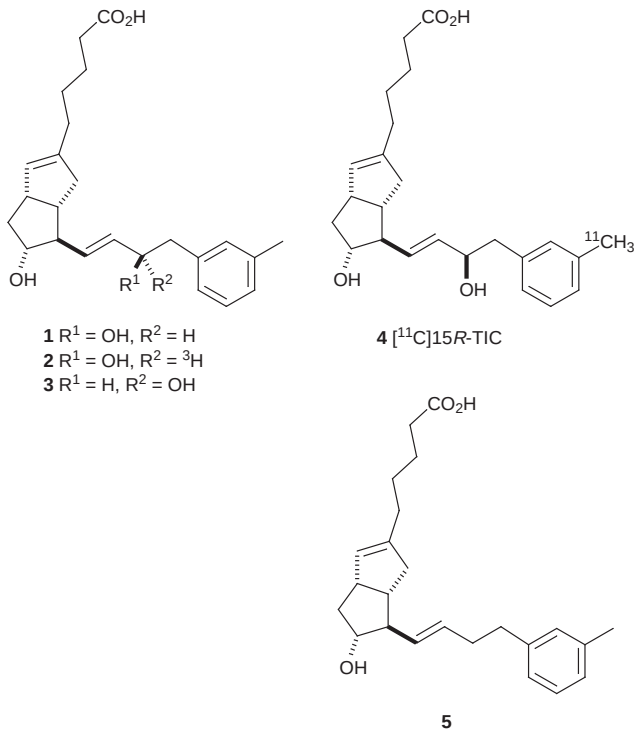
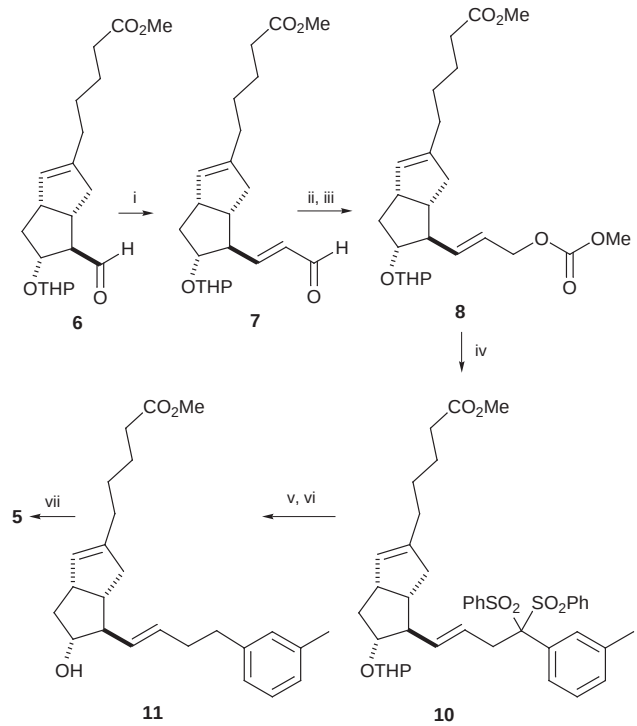
Biologically remarkable 15-deoxy-TIC has been realised by the removal of the C(15) chiral centre in 15*R*-TIC, a stable ligand for a CNS-type prostacyclin receptor (IP_2); this deoxy derivative exhibits ten-fold higher affinity and selectivity than 15*R*-TIC for the IP_2 receptor in correlation with the anti-apoptotic activity for neuronal cells.

In response to the increasing requirement for the development of specific molecular probes for the elucidation of the role of prostaglandin (PG) in brain functions,[1] we have designed (15*R*)-16-(*m*-tolyl)-17,18,19,20-tetranorisocarbacyclin **1** (15*R*-TIC) which has high binding affinity and selectivity for a novel prostacyclin (PGI_2) receptor subtype (IP_2) in the central nervous system (CNS).[2] This was utilised to visualise the specific location of the IP_2 receptor for both *in vitro* and *in vivo* systems by autoradiography of rat brain slices[3] and the positron emission tomography (PET) of a living rhesus monkey[4] using tritium-labelled ligand **2** and ^{11}C-labelled ligand **4**,[5] respectively.

The *R* configuration of C(15) in **1** was surprising, because the configuration of this hydroxy-bearing chiral centre in hormonal PGs is generally *S*.[6] In addition, 15*S*-TIC **3**, a C(15) stereoisomer of **1**, exhibited high binding affinity for both PGI_2 receptors, IP_1 and IP_2, as peripheral and CNS types, respectively.[2,3] These findings led us to speculate that the configuration of hydroxy-bearing C(15) in **1** may not be crucial for the binding with the IP_2 receptor and, therefore, that a common structure capable of more specifically recognising the IP_2 receptor could be hidden between these tolylisocarbacyclin structures, **1** and **3**. Accordingly, our interest has been directed to elucidating the essential structural elements necessary to discriminate between the two receptors, particularly focusing on the elimination of the C(15) chirality. Described herein is the synthesis of a 15-deoxy-TIC derivative with superb biological properties.

The title compound **5**, among other synthesised derivatives, exhibited the highest binding affinity and selectivity for the IP_2 receptor. As outlined in Scheme 1, the synthetic strategy consists of the stepwise construction of the *E*-olefin in the ω side-chain instead of the one-step Julia coupling,[7] because the starting aldehyde **6** is unstable under strongly basic conditions.[8] Thus aldehyde **6**[9] was condensed with (formylmethylene)-triphenylphosphorane in benzene at 80 °C to give (*E*)-α,β-unsaturated aldehyde **7** in 64% yield. Carbonyl reduction of **7** using $NaBH_4$ in MeOH, followed by methoxycarbonylation of the resulting allylic alcohol with $ClCO_2Me$ in the presence of





Scheme 1 *Reagents and conditions*: i, $Ph_3PCHCHO$, benzene, reflux; ii, $NaBH_4$, MeOH; iii, $ClCO_2Me$, DMAP, CH_2Cl_2; iv, $Pd_2(dba)_3$, $CHCl_3$, dppe, *m*-$CH_3C_6H_4CH(SO_2Ph)_2$ **9**, THF, 50 °C; v, Mg, MeOH; vi, TsOH, MeOH; vii, aq. NaOH, MeOH.

DMAP in CH_2Cl_2 gave the allyl carbonate **8** in 91% overall yield. The cross-coupling between the allyl carbonate **8** and the sulfone **9** was conducted at 50 °C in THF in the presence of a catalytic amount of $Pd_2(dba)_3$/dppe (1:2 ratio),[10] giving the sulfone **10** in 97% yield.† Reductive removal of the $PhSO_2$ group in **10** with Mg metal in MeOH and subsequent deprotection of the tetrahydropyranyl group with TsOH in MeOH gave the 15-deoxy derivative **11** in 84% overall yield. Finally, alkaline hydrolysis of **11** afforded the desired 15-deoxy-16-(*m*-tolyl)-17,18,19,20-tetranorisocarbacyclin, referred to as 15-deoxy-TIC **5**, in 94% yield.‡

The binding assay was conducted for the neuronal PGI_2 receptor proteins in frozen tissue sections containing the thalamus and the nucleus tractus solitarius (NTS) as representative of the IP_2 and IP_1 receptors, respectively, using C(15) tritium-labelled 15*S*-TIC ([^{3}H]15*S*-TIC)[2] as a standard radioligand. The 50% inhibitory concentrations of the binding (IC_{50} values) were determined by the degree of displacement of 10 nM [^{3}H]15*S*-TIC bound to each receptor by nonradioactive TIC derivatives. Accordingly, 15-deoxy-TIC **5** binds with the CNS-type IP_2 receptor (IC_{50} value = 3 nM) ten times more strongly than 15*R*-TIC **1** while maintaining the weak binding for IP_1 receptor (IC_{50} value = 1 μM).§ As a result, the binding selectivity for the IP_2 receptor [$IC_{50(IP1)}/IC_{50(IP2)}$] increased by a factor of 10. The importance of the length of the ω side-chain, the tolyl group and 13,14 double bond was clarified by a study of the structure binding affinity relationships. Thus the elimination of the hydroxy group at C(15) markedly enhanced the binding affinity and selectivity.

Since the discovery of 15*R*-TIC **1**,[2] we have pursued the biofunction of various TICs in the brain and developed CNS-specific PGI_2 ligands that exhibit neuronal survival-promoting activity both *in vitro* and *in vivo*.[11] The difference of the potency of the binding affinity between **1** and **5** is actually correlated well with the biological activity. Thus 15-deoxy-TIC **5** showed an inhibitory effect on apoptosis of neuronal cells induced by high oxygen (50%) atmosphere at ten-fold lower concentration than 15*R*-TIC **1** (IC_{50} = 30 and 300 nM, respectively). It should be noted that **5** did not exhibit any significant inhibitory effect on platelet aggregation, up to 400 nM, while PGI_2 derivatives which bind to the IP_1 receptor showed a very potent inhibitory effect at several nM.¶

Overall, we have found the essential structure that has conspicuous biological properties by rational molecular modification. The structural simplification by removal of the C(15) chirality will increase chemical or biological tolerance[12] to give a promising anti-apoptotic agent, particularly for chemotherapy of CNS neurons damaged with oxidative stress and ischemia.[11] Future progress will be reported in due course.

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Notes and references

† The sulfone **9** was prepared in 60% overall yield by mixing of *m*-methylbenzaldehyde and PhSH in the presence of TMSCl in CH_2Cl_2 (25 °C, overnight) followed by treatment with Oxone in aq. MeOH (25 °C, 5 h). *Selected data* for **9**: δ_H (270 MHz, $CDCl_3$) 2.22 (s, 3, CH_3), 5.40 (s, 1, CH), 7.1–7.2 (m, 4, arom.), 7.45 (t, 4, *J* = 7.4, arom.), 7.62 (t, 2, *J* 7.4, arom.), 7.79 (d, 4, *J* 7.4 Hz, arom.); δ_C (67.5 MHz, $CDCl_3$) 21.3, 88.7, 125.5, 128.6, 128.8, 129.8, 131.3, 134.5, 138.0, 138.6.

‡ *Selected data* for **5**: δ_H (270 MHz, $CDCl_3$) 1.4–2.1 (m, 10, 4 CH_2, CH, OH), 2.2–2.5 (m, 10, 2 CH_2, CH_3, CH_2CO, CH), 2.6–2.8 (m, 2, benzylic CH_2), 2.90–3.05 (br, 1, allylic CH), 3.63 (dt, 1, *J* 6.9, 9.4, CHO), 5.23 (dd, 1, *J* 8.9, 15.3, vinylic in chain), 5.28 (s, 1, vinylic in ring), 5.53 (dd, 1, J 6.9, 15.3, vinylic in chain), 6.97 (d, 1, *J* 7.9, arom.), 6.98 (s, 1, arom.), 7.00 (d, 1, *J* 7.4, arom.), 7.17 (t, 1, *J* 7.7, arom.); δ_C (67.5 MHz, $CDCl_3$) 21.4, 24.4, 27.1, 30.5, 33.6, 34.5, 35.8, 39.3, 39.6, 44.3, 45.6, 58.6, 77.9, 125.6, 126.5, 128.1, 128.4, 129.4, 132.1, 132.2, 137.8, 141.3, 141.8, 178.3.

§ The displacement of [^{3}H]15*R*-TIC **2** binding with the IP_2 receptor by **5** gave a similar result.

¶ The biological activity was examined at Teijin Co.

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