Eur J Med Chem (1992) 27, 155–159

Short communication

Synthesis and anti-aggregative activity of novel ω-achiral carba-analogues of prostacyclin

M Lopp[1], G Kobzar[1], M Bergmann[1], T Pehk[2], A Lopp[1], T Valimäe[2], M Viigimaa[3], Ü Lille[1]

[1]*Department of Prostanoid Chemistry, Institute of Chemistry, Akadeemia tee 15, 200108 Tallinn;*
[2]*Department of Physics, Institute of Chemical Physics and Biophysics, Lenini pst 10, 200 001 Tallinn;*
[3]*Department of Cardiology, Tartu University, Ülikooli 18, 202400 Tartu, Estonia*

(Received 7 December 1990; accepted 3 June 1991)

Summary — Novel stable bicyclo[3.3.0]octanic and bicyclo[4.2.0]octanic 13,14-didehydrocarbacyclins **1a–c**, **2a** bearing an achiral cyclohexanoic group at C-14 were synthesized. These analogues have been characterized by ^{13}C NMR spectroscopy. Compounds **1a–c** and **2a** were tested on rabbit and human platelet-rich blood plasma and **1a–c** on rat stomach and guinea pig trachea smooth muscles. E-isomers of **1a–b** were found to be less active but more selective than PGE_1. The anti-aggregative potency of E-isomer of compounds **1a–b** and Z-isomer of **2a** on human platelets was 10^{-1}–10^{-2} of the activity of PGE_1. The contractive activity of bicyclo[3.3.0]octane analogues **1a–c** was 10^{-3}–10^{-4} of that for PGE_1. On platelets and guinea-pig trachea 5E-isomers of the corresponding analogues were more potent, whereas on rat stomach muscle 5Z-isomers were.

ω-achiral carbacyclins / inhibitor of platelet aggregation / contractive activity / tissue selectivity of carbacyclins / ^{13}C NMR spectra of ω-achiral carbacyclins

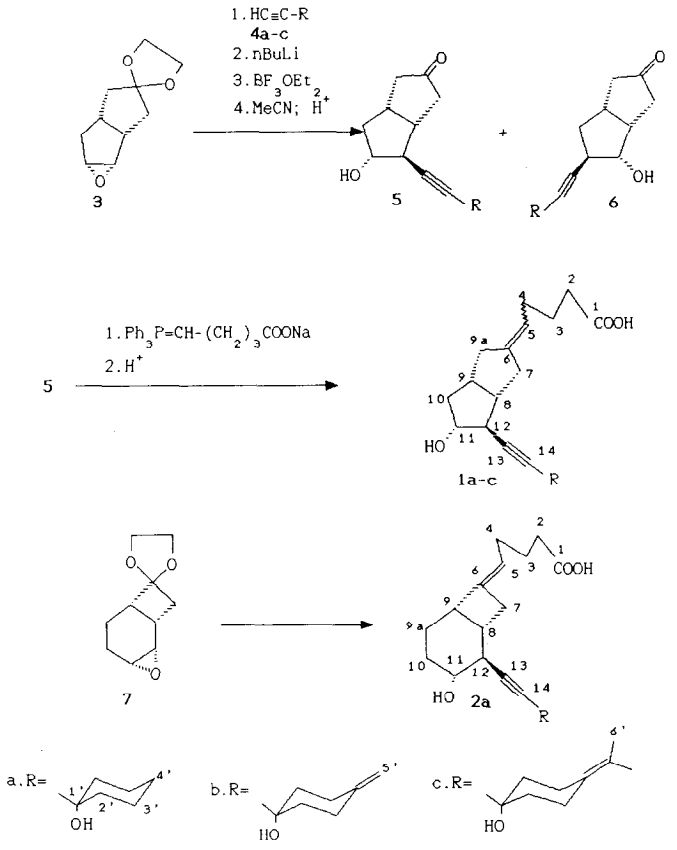
Introduction

Shortly after the discovery of prostacyclin [1] it became obvious that its high anti-aggregative ability on blood platelets combined with vasodilatation may have an important therapeutic use. At the same time, the use of the sodium salt of prostacyclin in medicine is limited by its instability in water solutions. These are the reasons why synthesis and investigation of different prostacyclin analogues were initiated. One of the strategies of the synthesis is based on the replacement of oxygen in unstable prostacyclin enol ether unit by carbon [2, 3]. These prostacyclin analogues are chemically sufficiently stable but undergo rapid enzymatic degradation *in vitro* [4]. Therefore, several other modifications that increase the metabolic stability have been prepared [5, 6]. Thus, it is known that the replacement of an *n*-amyl group at C-15 in the ω-chain by carbocyclic group enhances the resistance of the molecule towards 15-dehydrogenase and ω-oxidase [7]. It is known also that the replacement of the 13,14-double bond by the 13,14-triple bond suppresses the action of 13,14-hydrogenase, but at the same time, does not lower the activity of the compound [8]. In all cases the configuration of the hydroxy function at C-15 is important in determining the activity of the analogue [9, 10]. Unfortunately, most of the methods used in the synthesis of prostaglandins result in the mixtures of isomers at C-15. The problem of these isomers can be completely avoided by constructing an achiral ω-chain. In this case, cyclohexanol and its 1,4-disubstituted homologues, in which the hydroxy function is located at a symmetrically substituted carbon atom, may well serve this purpose.

In order to study the effect of replacing C-15 chiral carbon by the achiral structures we have synthesized 3 achiral 14-cyclohexyl analogues **1a–c** of known highly active 15-cyclohexyl carbacyclin derivative [11] and also one 14-cyclohexyl-bicyclo[4.2.0]-octane analogue **2a** of the corresponding 15-cyclohexyl analogue [12].

Chemistry

The synthesis of compounds **1a–c** (scheme 1) is based on epoxide opening in ethylene ketal 2,3-epoxy-bicyclo[3.3.0]-octane-7-one **3** [13] by alkynyllithium/BF_3Et_2O complex used by us previously for the synthesis of different prostaglandin and prostacyclin



Scheme 1.

analogues [13–15], the synthesis of **2a** from ethylene ketal of 2,3-epoxy-bicyclo[4.2.0]octane-7-one **7** is similar to that described in [12].

The acetylenic cyclohexanols **4a–c** were synthesized from the corresponding ketones using the Grignard reagent, and protected as trimethylsilyl ethers by using trimethylsilylimidazole in THF. The addition of borate complexes to epoxide **3** proceeds smoothly in THF at –78°C and leads, after removing the protecting group, to ketonediols **5** in 25–43% yield together with the corresponding regio-isomers **6** in the same amount. These isomers were separated by chromatography on silica gel. The target analogues **1a–c** were gained from ketondiols **5** by the Wittig reaction with an overall yield of *E/Z* isomers of 30–47%. The separation possibilities of *E/Z* isomers of carbacyclins have been discussed in [16]. The structure of the analogues was confirmed by ^{13}C NMR according to [17–19] and the chemical shifts are presented in table I.

Pharmacological results and discussion

The pharmacological activity of the carbacyclin analogues was studied by the following assays: 1) the inhibition of ADP-induced platelet aggregation was measured on human and rabbit blood platelet rich plasma (**1a–c**; **2a**); 2) the ability to induce smooth muscle contraction was determined on rat stomach and guinea-pig trachea preparations (**1a–c**). The results are presented in tables II and III. It is known that the 5*E*-isomers of bibyclo[3.3.0]octane analogues [7] and 5*Z*-isomers of bicyclo[4.2.0]octane analogues [12] of prostacyclin having a geometry similar to that of natural PGI_2 are considerably more potent inhibitors of platelet aggregation that their corresponding geometrical isomers. Our results on both human and rabbit blood platelets are in good agreement with this. It can be seen that the more potent the former isomer bearing the 'prostacyclin-like' α-chain is, the more noticeable is the difference in the activity of the 2 isomers. The potency of all less active 'prostacyclin-unlike' analogues is of the same magnitude. It means that the activity of these analogues is not sensitive to changes in the prostacyclin ω-chain. At the same time, it is interesting to note that for bicyclo[3.2.0]heptane analogues of prostacyclin the most potent are 5*E*-isomers with 'prostacyclin-unlike' structure [14].

If we compare the activity of ω-achiral 14-cyclohexyl analogues **1a–c** with the activity of known 15-cyclohexyl analogues [12, 20], it can be seen that the former are less potent. This is probably because the bulky 14-cyclohexyl group directly attached to the hydroxyl group hinders the interaction of the hydroxyl group with the active site of the receptor much more than the 15-cyclohexyl group in an α-position does. Of the compounds studied the most bulky analogue **1c** is the least active. The loss of chirality may be even less important. Nevertheless, compounds **1a** and **1b** (*E*-isomers) are still moderately active (respective EC_{50} values 5.1 x 10^{-7} M and 6.4 x 10^{-7} M) as inhibitors of human platelet aggregation so that they can be considered potent prostacyclin analogues.

On smooth muscle preparations, guinea-pig trachea was more sensitive than rat stomach to the contractive action of PGE_1 and also compounds **1a–c** (absolute EC_{50} values ≈ 10^{-5}–10^{-6} M). As in the case of blood platelets, on guinea-pig trachea muscle 5*E*-isomers of **1a–c** induced response at lower concentrations than the corresponding 5*Z*-isomers. On rat stomach muscle, however, the latter were more active. This fact allows us to assume the possible difference between the receptors mediating the effects of prostacyclin analogues in smooth muscles of different origin.

It is known that cell membranes can have several specific binding sites for different prostaglandins and

Table I. ^{13}C NMR shifts (δ, ppm downfield from internal tetramethylsilane) of carbacyclin analogues **1a–c**, **2a**.

Carbon	*1a*		*1b*		*1c*		*2a*	
	E	*Z*	*E*	*Z*	*E*	*Z*	*E*	*Z*
1	177.9	178.0	177.4	177.1	178.1	178.2	178.6	178.7
2	33.3	33.3	31.6	31.5	33.2	33.2	33.4	33.4
3	24.6	24.7	24.7	24.7	24.6	24.6	24.5	25.0
4	28.6	28.5	28.6	28.5	28.5	28.5	26.8	26.8
5	121.5	121.4	121.6	121.6	121.5	121.4	117.9	121.2
6	142.0	142.2	147.2	147.2	141.9	142.1	141.2	140.7
7	35.6	33.0	35.6	33.3	35.5	33.1	35.2	35.9
8	46.6	47.5	46.7	47.5	46.5	47.4	37.4	35.8
9	37.5	36.8	37.6	36.7	37.4	36.7	42.2	41.6
9a	38.7	40.9	38.7	40.8	38.6	40.8	22.5	23.6
10	40.9	40.5	41.1	40.6	40.9	40.4	29.1	29.8
11	78.1	78.2	78.2	78.1	78.0	78.1	71.5	71.3
12	44.7	45.6	44.7	45.5	44.7	45.5	41.1	41.2
13	85.3	85.7	84.8	84.9	84.9	85.1	85.4	85.1
14	85.8	86.7	86.1	86.1	85.8	85.7	86.3	86.1
1'	68.9	68.9	68.3	68.3	68.9	68.9	68.7	68.7
2'	40.1	40.1	40.8	40.8	40.6	40.6	40.0	40.0
3'	23.5	23.5	31.6	31.5	26.4	26.4	23.5	23.5
4'	25.3	25.3	141.9	142.0	129.2	129.2	25.2	25.2
5'			108.1	108.1	121.8	121.8		
6'					20.1	20.1		

that, on the other hand, different prostaglandins can activate the same receptor [21]. So, PGI_2 and its analogues can activate not only IP but also EP_1 receptors [22]. It is generally accepted that PGE_1 and PGI_2, when inhibiting platelet aggregation, act on the same IP receptors [23, 24] and there is no convincing evidence for the presence of EP_1 receptors on platelets [24]. Therefore, prostacyclin analogues can activate only IP receptors on platelets. On the contrary, stomach and tracheal muscles have EP_1 receptors [21, 22] and the contractive effects of the analogues studied in present work can also be mediated through these receptors.

Table II. Relative anti-aggregative potencies of prostacyclin analogues in human and rabbit blood platelet-rich plasma. PGE_1 $EC_{50} = 4.0 \pm 0.5\ 10^{-8}$ M; $EC_{50} = 6.2 \pm 0.8\ 10^{-8}$ M.

Compound	*Man*	*Rabbit*
PGE_1	1	1
1a 5*E*-isomer	7.8×10^{-2}	4.4×10^{-3}
5*Z*-isomer	1.0×10^{-3}	$< 6.2 \times 10^{-4}$
1b 5*E*-isomer	6.3×10^{-2}	6.2×10^{-3}
5*Z*-isomer	2.1×10^{-3}	$< 6.2 \times 10^{-4}$
1c 5*E*-isomer	3.1×10^{-3}	9.3×10^{-4}
5*Z*-isomer	1.3×10^{-3}	$< 6.2 \times 10^{-4}$
2a 5*Z*-isomer	8.9×10^{-3}	2.7×10^{-3}
5*E*-isomer	8.0×10^{-4}	9.3×10^{-4}

If we compare the relative anti-aggregative and contractive activities of the more potent 5*E*-isomers of compounds **1a–b** (tables II and III) it can be seen that being less active than PGE_1 they act more selectively on different tissues. So, the relative anti-aggregative activity of these compounds is 10^{-1}–10^{-2}, but the relative contractive activity is only 10^{-3}–10^{-4} of that of PGE_1.

Experimental protocols

Chemistry

IR spectra were recorded for KBr discs on a Specord IR 75 spectrometer. ^{13}C NMR spectra were obtained on a Bruker

Table III. Relative potencies of prostacyclin analogues on rat stomach and guinea pig tracheal muscles. PGE_1 $EC_{50} = 1.9 \pm 0.7\ 10^{-8}$ M; $EC_{50} = 1.7 \pm 0.3\ 10^{-9}$ M.

Compound	*Rat stomach muscle*	*Guinea pig tracheal muscle*
PGE_1	1	1
1a 5*E*-isomer	1.4×10^{-3}	5.8×10^{-4}
5*Z*-isomer	1.5×10^{-3}	1.2×10^{-4}
1b 5*E*-isomer	1.5×10^{-3}	7.0×10^{-4}
5*Z*-isomer	4.3×10^{-3}	3.6×10^{-4}
1c 5*E*-isomer	1.2×10^{-3}	8.5×10^{-4}
5*Z*-isomer	4.3×10^{-3}	3.2×10^{-4}

AM-500 spectrometer in $CDCl_3$ solution. The chemical shifts are reported relative to tetramethylsilane. All chemical synthesis procedures were carried out in a flame dried apparatus under Ar atmosphere. THF was dried on the KOH granules and distilled from $LiAlH_4$ shortly before use. BF_3OEt_2 (Fluka) was used without purification. Column chromatography was performed with silica gel L 40-100 (Chemapol), TLC with DC-Alufolien Kieselgel 60-F_{254} and visualized by phosphor-molybdenic acid (5%) in ethanol. HPLC analysis of the compounds was carried out on a Du Pont LC 8843 chromatograph equipped with UV spectrophotometric and refractometric detectors. All the compounds were analyzed for C, H and the analytical values are given as ± 0.4% of the theoretical values. All compounds were obtained as racemic mixtures.

5E- and 5Z -9a-Carba-14(1-hydroxycyclohexyl)-13,14-dehydro prostaglandin I_2 ***1a***

To cyclohexanone (2.94 g, 30 mmol) ethynylmagnesium bromide in THF (2.78 ml, 39 mmol) at –15°C was added and the mixture was stirred at room temperature for 5 h. 5% HCl was added until pH ≈ 4–5 and the product was extracted with ether. After purification on silicagel (50 g, benzene acetone 10:1) 2.3 g of 2(1-hydroxycyclohexyl)-eth-1-yne was obtained. A part of this alcohol (372 mg, 3 mmol) was silylated with TSIM (0.88 ml, 6 mmol) in THF (5 ml) and purified on silica gel (10 g, hexane). A mixture of 2(1-trimethylsilyloxy-cyclohexyl)-eth-1-yne (455 mg, 2.3 mmol) in THF (3 ml) was cooled to –78°C and nBuLi (2.3 mmol in hexane) was added. The mixture was stirred for 10 min and then BF_3OEt_2 (0.3 ml, 2.8 mmol) was added and stirring was continued for 10 min. Ethylene ketal of 2,3-epoxy-bicyclo[3.3.0]octane-7-one **3** (265 mg, 1.46 mmol) in THF (1.2 ml) was added. The mixture was stirred at –78°C for 1 h and then an aqueous NH_4Cl solution (10 ml) was added. The mixture was warmed to room temperature and subjected to normal work-up. A normal work-up procedure of the reaction mixture consisted of 3 extractions with ethyl acetate, washing with brine, drying with $MgSO_4$, and the removal of the solvent on a rotary evaporator. The product was deblocked in a mixture of MeCN (38 ml), 0.2 N H_2SO_4 (3.8 ml) and H_2O (15 ml) for 2 h, neutralized with a $NaHCO_3$ solution to pH ≈ 7, the solvent was removed and the residue was subjected to usual work-up. After chromatographic separation of isomers ketone **5** (153 mg) and ketone **6** (160 mg) were obtained. Ketone **5** was silylated with trimethylsilyl-imidazole (0.5 ml) in THF and subjected to the Wittig reaction with $Ph_3P{=}CH(CH_2)_3COONa$ (prepared from 375 mg of Ph_3P^+-$(CH_2)_4COOHBr^-$) in DMSO. After HPLC separation of products **1a** *E*-isomer (31 mg oil ^{13}C NMR in table I. IR: 960, 1060, 1700, 2240, 3360 cm^{-1}. Anal $C_{21}H_{29}O_4$ (C, H)) and **1a** *Z*-isomer (29 mg oil ^{13}NMR in table I. IR: 1060, 1700, 2240, 3360 cm^{-1}. Anal $C_{21}H_{29}O_4$ (C, H)) was obtained.

5E- and 5Z- 9a-Carba-14(1-hydroxy-4-methylidenecyclohexyl)-13,14-dehydro prostaglandin I_2 ***1b***

According to the procedure described above **1b** 5*E*-isomer (27 mg oil ^{13}C NMR in table I. IR: 890, 960, 1060, 1700, 2240, 3360 cm^{-1}. Anal $C_{22}H_{29}O_4$ (C, H)) and **1b** 5*Z*-isomer (24 mg oil ^{13}C NMR in table I. IR: 890, 1060, 1700, 2240, 3360 cm^{-1}. Anal $C_{22}H_{29}O_4$ (C, H)) were prepared from 2(1-trimethylsilyl-oxy-4-methylene-cyclohexyl)-eth-1-yne (591 mg), epoxide **3** (345 mg, $Ph_3P^+(CH_2)_4COOHBr^-$ (485 mg).

5E- and 5Z- 9a-Carba-14(1-hydroxy-4-isopropylidene cyclohexyl)13,14-dehydro prostaglandin I_2 ***1c***

According to the procedure described above **1c** 5*E*-isomer (27 mg oil ^{13}C NMR in table I. IR: 960, 1060, 1600, 2240, 3360 cm^{-1}. Anal $C_{24}H_{33}O_4$ (C, H)) and **1c** 5*Z*-isomer (20 mg oil. ^{13}C NMR in table I. IR: 1060, 1600, 2240, 3360 cm^{-1}. Anal $C_{24}H_{33}O_4$ (C, H)) were prepared from 2(1-trimethylsilyloxy-4-isopropylidene-cyclohexyl)-eth-1-yne (610 mg), epoxide **3** (340 mg) and $Ph_3P^+(CH_2)_4$-$COOHBr^+$ (333 mg).

5E- and 5Z-Isomers of 2-ekso(2[1-hydroxycyclohexyl]-1-ethynyl)-3-endo-hydroxy-7(5-carboxy-1-pentylidene)-bicyclo[4.2.0]-octane ***2a***

According to the procedure described in [12], **2a** 5*E*-isomer (53 mg oil, ^{13}C NMR in table I. Anal $C_{21}H_{30}O_4$ (C, H)) were prepared from 2(1-trimethylsilyloxycyclohexyl)eth-1-yne (950 mg), epoxide **7** (650 mg) and $Ph_3P^+(CH_2)_4COOHBr^-$ (983 mg).

Pharmacological methods

Inhibition of platelet aggregation. This was assessed by the Born turbidimetric method [25]. Fresh blood from a local blood bank or from rabbits was collected in plastic vessels containing trisodium citrate (3.8% v/v, 0.1 vol) and centrifuged at 160 *g* for 10 min at room temperature. The platelet-rich plasma (PRP) was decanted into plastic tubes and kept at room temperature. Inhibition of platelet aggregation was determined on Chrono-Log aggregometer at 37°C. Prostacyclin analogs were added to 0.5 ml of PRP in 1 min prior of the addition of sufficient amount of ADP (1–5 mM in the case of human blood and 5–20 mM in the case of rabbit blood) to cause non-reversing aggregation.

Contractions of smooth muscles. Strips from stomach fundus of male rats were prepared by the method of Vane [26]. Preparations of trachealis muscle were taken from guinea-pigs of either sex. The lowest part of the trachea containing 2–3 bands of cartilage was excised and cut through the center of the cartilage. The strips were suspended in a 10-ml organ bath containing the Krebs solution maintained at 37°C and gassed with a O_2 and CO_2 mixture (95:5). A resting tension of 1 g was applied. The changes in muscle tension were recorded isometrically. Agonist concentration–response curves were obtained cumulatively. Experiments with tracheal muscle were carried out in the presence of indomethacin (1 mg/ml).

Expression of potency. EC_{50} for each compound was defined as the concentration producing 50% of its own maximum response and was calculated from concentration–response curves. The values of EC_{50} quoted are arithmetic means of at least 3 determinations. The value of EC_{50} for PGE_1 was equal to 1. The relative potencies were determined as a ratio of the value of standard agonist (PGE_1) to those for prostaglandin analog. PGE_1 was chosen as a standard agonist because of instability of PGI_2 and its sodium salt in water solutions [27].

Drugs and solutions. The composition of the Krebs solution (g/l) was as follows: NaCl 5.54; KCl 0.35; $NaHCO_3$ 2.1; $CaCl_2$ 0.28; $MgSO_4$ $7H_2O$ 0.29; KH_2PO_4 0.16; glucose 2.1. The following drugs were used: indomethacin (Sigma); ADP (Chrono-log); PGE_1 (Pilot-Production Plant of the Institute of Chemistry, Tallinn). PGE_1 and prostacyclin analogs were dissolved in ethanol and diluted shortly before use with a 0.9% NaCl solution. All the solutions measured were kept on ice before use.

Acknowledgments

The authors wish to thank Mrs Liidia Lahe for performing IR spectra, Mr Madis Lõhmus for HPLC analysis and Mr Tõnis Kanger for assistance during preparation the manuscript.

References

1 Moncada S, Gryglevski RJ, Bunting S, Vane JR (1976) *Nature* 263, 663–665
2 Kojima K, Sakai K (1978) *Tetrahedron Lett* 19, 3743–3746
3 Nicolaou KC, Sipio WJ, Magolda RL, Seitz Z, Barnette WE (1978) *J Chem Soc Chem Commun* 24, 1067–1068
4 Aiken JW, Shebuski RJ (1980) *Prostaglandins* 19, 629–638
5 Floche L, Böhlke H, Frankus E, Kim SMA, Lintz W, Locsheng G, Michel K (1983) *Arzneim Forsch* B33, 1240–1248
6 Aristoff PA (1985) Synthesis of prostacyclin analogs. *In: Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Pike JE, Morton GR, eds) Raven Press, NY, 14, 309–392
7 Whittle BJR, Moncada S (1985) *Circulation* 73, 1219–1225
8 Fried J, Lin CH (1973) *J Med Chem* 16, 429–430
9 Ramwell PW, Shaw JE, Corey EJ (1969) *Nature* 222, 1251–1253
10 Corey EJ, Terashima S, Ramwell PW, Jessup R, Weinshenkr NM, Floyd DM, Crosby GA (1972) *J Org Chem* 37, 3043–3044
11 Monginelli N, Gandolfi C DE 3,335,389; CA 101, 151670y
12 Kluge AF, Kertesz DJ, O-Yang C, Wu HY (1987) *J Org Chem* 52, 2860–2868
13 Parve O, Pals A, Lõhmus M, Välimäe T, Lahe L, Lopp M, Lille Ü (1985) *Eesti NSV TA Toimetised Keemia* 34, 285–291
14 Lopp M, Müraus A, Parve O, Välimäe T, Lopp A, Lille Ü (1988) *Biorg Khim* (in Russian) 14, 222–231
15 Lopp M, Paju A, Pehk T, Lille Ü (1969) *J Chem Res (S)* 210–211
16 Lõhmus M, Lopp M, Lille Ü, Veisserik J (1988) *J Chromatography* 450, 105–109
17 Cooper GF, Fried J (1973) *Proc Natl Acad Sci USA* 70, 1579–1584
18 Pehk T, Välimäe T, Samel N, Lopp M, Lille Ü, Lippmaa E (1982) *Eesti NSV TA Toimetised Keemia* 31, 85–90
19 Välimäe T, Pehk T, Lippmaa E, Lopp M, Lille Ü (1986) *Eesti NSV TA Toimetised Keemia* 35, 165–192
20 Tierauch KH, Stüzzebecher CSt, Schillinger E, Radüchel W, Skuballa W, Vorbrüggen H (1988) *Prostaglandins* 35, 855–868
21 Kennedy I, Coleman RA, Humphrey PPA, Levy GP, Lumley P (1982) *Prostaglandins* 24, 667–689
22 Dong YJ, Jones RL, Wilson NH (1986) *Br J Pharmacol* 87, 97–107
23 Whittle BJR, Moncada S, Vane JR (1978) *Prostaglandins* 16, 373–388
24 Giles H (1990) *Trends Pharmacol Sci* 11, 301–304
25 Born GVR (1962) *Nature* 194, 927–929
26 Vane JR (1957) *Br J Pharmacol* 12, 344–349
27 Moncada S, Vane JR (1979) Prostacyclin formation and effects. *In: Chemistry, Biochemistry and Pharmacological Activity of Prostanoids* (Roberts SM, Scheimann F, eds) Pergamon Press, Oxford, 258–273