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SYNTHESIS OF CHEMICALLY STABLE 9-SUBSTITUTED CARBACYCLIN DERIVATIVES AND THEIR BIOLOGICAL USE

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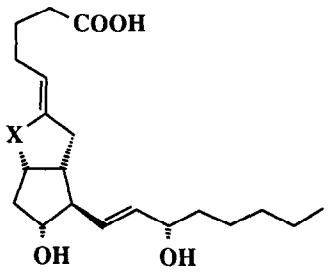
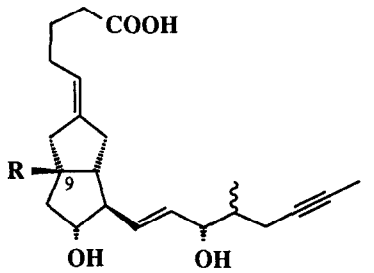
SUMMARY: A variety of 9-substituted carbacyclin derivatives was synthesized and their biological properties evaluated. It is shown that a triple bond directly attached to the bicyclo[3.3.0]system resulted in biologically very active derivatives, one of which was successfully used to establish a highly selective and sensitive radio-immuno-assay (RIA) for Iloprost.

INTRODUCTION AND CONCEPT: The discovery of prostacyclin (PGI_2, **1a**)[1] as an important endogenous compound with strong blood platelet inhibitory and vasodilatory action gave a strong impetus to chemical and cardiovascular research. Due to the labile enol ether moiety and the intramolecular acid catalysis of the carboxylic group, PGI_2 is hydrolized nonenzymatically in the blood with a half life of about 3 minutes[2]. Consequently, many efforts were made to synthesize chemically stable and biologically potent analogs of PGI_2 for potential clinical application. In one of these approaches, the enol ether oxygen in PGI_2 was replaced by a methylene group, leading to carbacyclin (**1b**)[3] which is chemically stable but possesses less than 10% of the biological activity of PGI_2[4]. Further structural modification in our own laboratory to increase the activity as well as the metabolic stability concentrated on the lower side chain and resulted in the synthesis of our stable carbacyclin analog Iloprost[5] which turned out to be biologically equipotent to PGI_2 and to be clinically very effective against a variety of cardiovascular diseases[5d].



1a Prostacyclin (X = O)

1b Carbacyclin (X = CH_2)



2 Iloprost (R = H)

With the powerful tool Iloprost in hand, we asked ourselves whether it is possible to synthesize derivatives of Iloprost which could be used

1. for irreversible binding to artificial surfaces e.g. for the development of thrombo-resistant synthetic vascular grafts with a reduced risk of thrombus formation after implantation,
2. for the development of a highly sensitive and specific RIA for the detection of Iloprost, and
3. for the isolation and purification of the PGI_2-receptor.

For all these purposes, we needed an additional functionalized chain connected to a position of the carbacyclin skeleton of Iloprost which would not reduce its high biological activity. Since it is well-known that modifications at the carboxylic group as well as structural changes in the lower side chain, will normally cause a loss in activity, we became interested in work by Aristoff et al.[6], who described the synthesis of carbacyclin derivatives substituted in position 9. They demonstrated that the introduction of an alkyl or acetylene group in the 9-position gave derivatives which are about as potent as PGI_2 itself. Thus, we turned our attention to the synthesis of 9-substituted carbacyclins with an ω-functionality at the 9-substituent, necessary for the attachment of the carbacyclin to proteins or polymers, without any decrease in biological activity.

SYNTHESIS: Starting with the optically active Corey-aldehyde **3**, we introduced the lower chain by a Wittig-Horner reaction using dimethyl-3(RS)-methyl-2-oxo-5-heptynylphosphonate, which produced a nearly 1:1 mixture of epimers at position 16 (prostaglandin numbering). Reduction of the α,β-unsaturated ketone **4** resulted in both epimeric alcohols **5** and **6** which were readily separated by chromatography. The alcohol **5** with the unnatural 15β-configuration could be recycled by oxidation-reduction in satisfactory yields to **6**. Deprotection of **6** in the 11-position and introduction of the THP-ether moiety in the 11- and 15-positions gave compound **8**. Opening of the five membered lactone in **8** using the two phase system ether/1N NaOH, subsequent acidification with citric acid, and rapid extraction at 0°C with chloroform yielded the sensitive hydroxy acid **9**, which was oxidized immediately to ketone **10** to avoid relactonisation. Intramolecular Wittig cyclization with the Bestmann reagent[7] produced **11**, the key intermediate for the introduction of our functionalized 9-substituent **12**, in good yields. 1,4-Addition of the acetylenic side chain according to J. Schwartz et al.[8] gave **13** in up to 60% yield. We found that reproducible results in this Ni(I) catalyzed reaction were obtained only if all traces of oxygen were carefully removed by repeated (5 to 10 times) evacuation and ventilation with argon. Wittig reaction of **13** under mild conditions using an excess of carboxybutyltriphenylphosphonium bromide, followed by esterification of the carboxylic acid and cleavage of the tert.-butyldiphenylsilylether, gave **16** as a mixture of E/Z isomers. The ω-functionality in the 9-substituent of **16** could now be readily manipulated as shown by the preparation of **17** to **19**. Cleavage of the protecting groups in **16** to **19** led finally to the unprotected mixture of 5-E/Z-isomers **24** to **30**, which were separated by chromatography[9] in all cases except for **26**.

Furthermore, starting from **27** or **28**, it was possible to introduce iodine or ^{125}iodine to afford **31** to **34**. Alternatively, it was also possible to oxidize the 11- and 15-hydroxy groups in **31** and then reduce the very labile 11,15-dioxo compound **35** with $NaBT_4$. The resulting tritium-labeled 11,15-diastereomers were separated subsequently by HPLC. Both methods introduce the radioactive labels at the very end of the synthesis.

BIOLOGICAL RESULTS: As shown in *Table 1*, a number of different 9-substituted carbacyclin derivatives was tested[10]. The receptor affinities to the PGI_2-receptor and the IC_{50}-values for the inhibition of blood platelet aggregation (human platelet-rich plasma) stimulated by ADP are compared with Iloprost (= 1

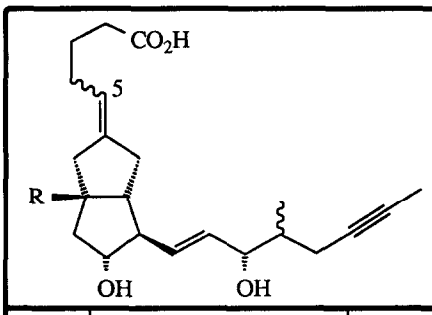
as standard).

To obtain highly potent derivatives, the triple bond in the 9-substituent must be located directly at the bicyclic ring system[6] and the ω-functionality in the 9-substituent has to be a non-polar group (N_3, Br, I).

The compound mixture **26** (entry 08, *Table 1*), was successfully used to develop a highly sensitive and specific radioimmunoassay (RIA) for the determination of Iloprost[11].

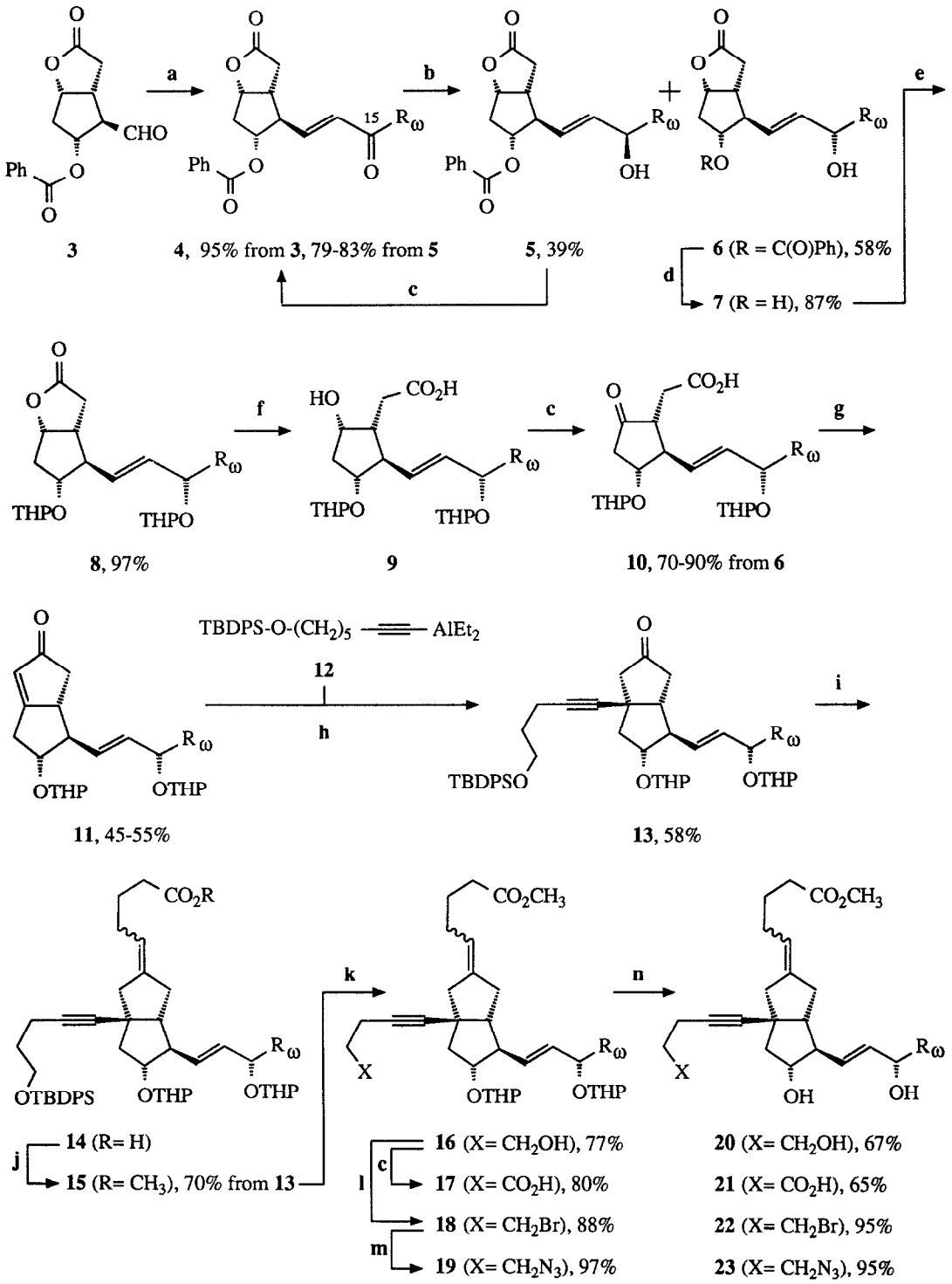
Although the azido compound **29** (entry 10) was coupled photochemically in an irreversible manner to the PGI_2-receptor within the platelet membrane, subsequent SDS-treatment did not give a labeled PGI_2-receptor-agonist-complex[12].

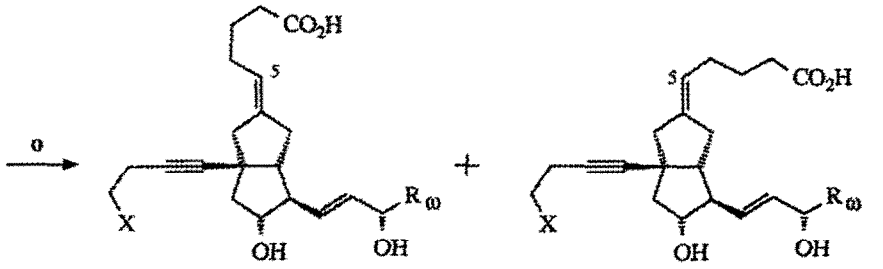
Table 1



Biological activities of 9-substituted carbacyclin derivatives

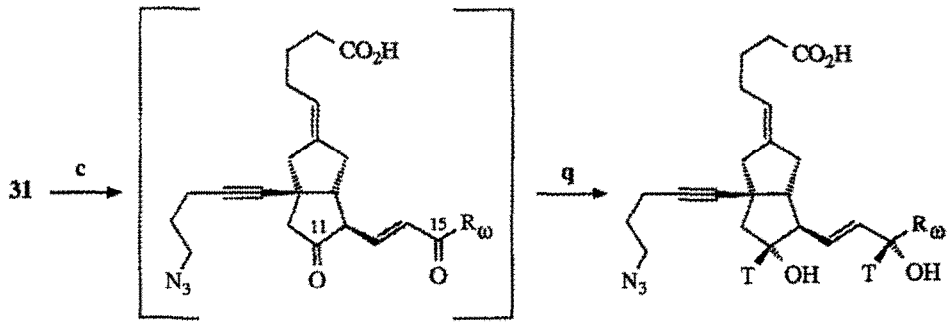
Entry	-R [13]	$^5\Delta^9$	$K_F(PGI_2)=\frac{K_D\text{-Test}}{K_D\text{-Iloprost}}$	inhibition of blood platelet aggregation induced by ADP (IC_{50})·10^{-9}M	relative potency to Iloprost
01	-H (Iloprost)	E	1.0	0.2-0.5	1.0
02	-H ("5Z-Iloprost")	Z	11	4400	0.0001
03	$-(CH_2)_5$-OH	Z	130	7000	0.0006
04		E	n.k.	11000	0.00004
05	$-(CH_2)_2-\equiv-(CH_2)_3$-OH	E/Z	180	400	0.002
06	$-\equiv-(CH_2)_3$-OH	Z	25	10	0.2
07		E	300	200	0.004
08	$-\equiv-(CH_2)_2$-COOH	E/Z	200	2000	0.0002
09	$-(CH_2)_5-N_3$	E/Z	29	500	0.0005
10	$-\equiv-(CH_2)_3-N_3$	Z	6.0	20	0.1
11		E	80	850	0.003
12	$-\equiv-(CH_2)_3$-Br	Z	3.0	0.5-2.5	1.0
13		E		100-250	0.001
14	$-\equiv-(CH_2)_3$-I	Z	5.6	5	1.0
15		E	>100	200	0.001


a
b
c
d
e
f
g
h
i
j
k
l
m
n
CHO
Ph
THPO
OTHP
OH
RO
HO
CO2H
TBDPS-O-(CH2)5
AlEt2
12
TBDPSO
OTBDPS
CO2R
CO2CH3
X
3
4, 95% from 3, 79-83% from 5
5, 39%
6 (R = C(O)Ph), 58%
7 (R = H), 87%
8, 97%
9
10, 70-90% from 6
11, 45-55%
13, 58%
14 (R= H)
15 (R= CH3), 70% from 13
16 (X= CH2OH), 77%
17 (X= CO2H), 80%
18 (X= CH2Br), 88%
19 (X= CH2N3), 97%
20 (X= CH2OH), 67%
21 (X= CO2H), 65%
22 (X= CH2Br), 95%
23 (X= CH2N3), 95%



R_ω: 

THP: Tetrahydropyranyl TBDPS: tert.-Butyl-diphenylsilyl



35 36

a: Dimethyl-3(RS)-methyl-2-oxo-5-heptynylphosphonate, NaH, DME, -45°C, 3h, argon; SiO_2; **b:** $NaBH_4$, MeOH, THF, -40°C, 3h, argon; SiO_2; **c:** Jones-oxidation, acetone, -20°C, argon; **d:** K_2CO_3, MeOH, rt, 5h, argon; SiO_2; **e:** dihydropyrane, cat. p-TsOH, CH_2Cl_2, rt, 0.5h, argon; SiO_2; **f:** 1N NaOH, THF, ether, rt, 24h; H^+ (pH 4-5); next step without purification; **g:** $Ph_3P{=}C{=}C{=}N\text{-}Ph$, ethyl acetate, rf, 3h, argon; toluene, EtOH, rf, 17h, argon; SiO_2; **h:** cat. Ni(I), ether, -10°C, 1.5h, argon; SiO_2; **i:** 6 eq. carboxybutyltriphenyl-phosphonium bromide, NaH-DMSO, toluene, 55°C, 6h, argon; next step without purification; **j:** CH_2N_2, ether, CH_2Cl_2, 3°C, 1h; SiO_2; **k:** 1M TBAF, THF, rt, 1h, argon; SiO_2; **l:** Ph_3P, CBr_4, collidine, CH_3CN, rt, 0.5h, argon; SiO_2; **m:** NaN_3, DMF, 60°C, 3.5h, argon; SiO_2; **n:** HOAc, H_2O, THF, rt, 16h; SiO_2; **o:** 5% LiOH, MeOH, rt, 1.5h; SiO_2; **p:** 50% NaI, acetone, rt, 3.5h; SiO_2; **q:** $NaBT_4$, MeOH, -45°C, 1h, HPLC.

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REFERENCES AND NOTES:

1) a) Moncada S., Gryglewski R., Bunting S., Vane J.R. *Nature* **1976** *263*, 663. b) Johnson, R.A.; Morton, D.R.; Kinner, J.H.; Gorman, R.R.; Mc Duire, J.C.; Sun, F.F.; Whittaker, N.; Bunting, S.; Salmon, J.; Moncada, S.; Vane, J.R. *Prostaglandins* **1976**, *12*, 915.

2) Moncada S., Vane J.R. *J. Med. Chem.* **1980**, *23*, 591.

3) a) Kojima, K.; Sakai, K. *Tetrahedron Lett.* **1978**, *39*, 3743. b) Nicolaou K.C.; Sipio, W.J.; Magolda, R.L.; Seitz, St.; Barnette, W.E. *J.C.S. Chem. Comm.* **1978**, 1067. see also: c) Aristoff, P.A. *J. Org. Chem.* **1981**, *46*, 1954.

4) a) Nickolson, R.C.; Town, M.H.; Vorbrüggen, H. *Med. Res. Rev.* **1985**, *5*, 1. b) Dombrovskii, V.A.; Gracheva, E.V.; Kochergin, P.M. *Russian Chem. Rev.* **1986**, *55*, 978.

5) a) For a review of the synthesis and pharmacological action of Iloprost, see: Prostacyclin and Its Stable Analogue Iloprost, Eds.: Gryglewski R.J., G. Stock; Springer Verlag 1987. b) Skuballa, W.; Schillinger, E.; Stürzebecher, C.-St.; Vorbrüggen, H. *J. Med. Chem.* **1986**, *29*, 313. c) Stürzebecher, C.-St.; Haberey, M.; Müller, B.; Schillinger, E.; Schröder, G.; Skuballa, W.; Stock, G.; Vorbrüggen, H.; Witt, W. *Prostaglandins* **1986**, *31*, 95. d) Oberender, H.; Krais, Th.; Schäfer, M.; Belcher, G. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **1989**, *19*, 311.

6) Aristoff P.A., Johnson P.D., Harrison A.W. *J. Org. Chem.* **1983**, *48*, 5341.

7) a) Bestmann H.J., *Angew. Chem.* **1977**, *89*, 361. b) Bestmann H.J., Schade G., Schmid G. *Angew. Chem.* **1980**, *92*, 856. c) Bennua B., Dahl H., Vorbrüggen H. Synthesis **1986**, 41.

8) a) Hansen R.T., Carr D.B., Schwartz J. *J. Am. Chem. Soc.* **1978**, *100*, 2244. b) Schwartz J., Carr D.B., Hansen R.T., Dayrit F.M. *J. Org. Chem.* **1980**, *45*, 3053. c) Schwartz J., Dayrit F.M., *J. Am. Chem. Soc.* **1981**, *103*, 4466.

9) The assignment of stereochemistry at C-5 is based upon biological data. It is assumed that the more potent isomer possesses the same configuration as PGI_2. Due to the priority rules, 5-E/Z is reversed in the 9-substituted carbacyclins.

10) Biological testing was conducted at Schering AG by Drs. K.-H. Thierauch and C.-St. Stürzebecher.

11) Hildebrand M., Nieuweboer B., Biere H., Klar U., Seemann G., Krause W., Jakobs U. *Eicosanoids* **1990**, *3*, 165.

12) We are indebted to Prof. MacDermot for his attempts to isolate the PGI_2-receptor.

13) The synthesis of compounds mentioned in entries 03 to 05 and 09 was performed in a similar way to the one shown in this paper.