Spirophostins: Conformationally Restricted Analogues of Adenophostin A

Martin de Kort,[a] Anouk D. Regenbogen,[a] A. Rob P. M. Valentijn,[a] R. A. John Challiss,[b] Yoriko Iwata,[c] Shuichi Miyamoto,[c] Gijs A. van der Marel,[a] and Jacques H. van Boom*[a]

Abstract: The synthesis, biological evaluation, and molecular modeling of two conformationally restricted analogues of adenophostin A (**1**), denominated as spirophostin (3*R*)-**10** and (3*S*)-**11**, as novel ligands for the D-*myo*-inositol 1,4,5-trisphosphate receptor (IP_3R), is presented. These diastereoisomeric spiroketals are synthesized by spiroketalization of D-glucose derivatives (2*S*)-**15** and (2*R*)-**16**, separation of the protected isomers (3*R*)-**19** and (3*S*)-**20**, followed by phosphorylation and deprotection. The spirophostins (3*R*)-**10** and (3*S*)-**11** display comparable biological activity, with a ^3H-IP_3-displacing and Ca^{2+}-releasing potency less than IP_3 and adenophostin A.

Keywords: adenophostin A · calcium release · carbohydrates · molecular modeling · spiro compounds

Introduction

The fungal metabolites adenophostin A and B (**1** and **2**, Figure 1),[1] which are full agonists of the D-*myo*-inositol 1,4,5-trisphosphate receptor (IP_3R), show potencies $\approx 10-100$ times higher than IP_3 (**4**). Interestingly, the observed 1000-fold reduced binding affinity[1c] of the 2′-dephosphorylated derivative **3**, indicates that the 2′-phosphate contributes significantly to the activity of adenophostin A (**1**). Moreover, molecular modeling studies[2, 3] showed that this phosphate occupies a slightly more remote position from the *trans*-3″,4″-bisphosphate than in IP_3 (**4**).

In order to get a better insight into the precise role of the 2′-phosphate and the adenine function in adenophostin A (**1**), several analogues have been synthesized and evaluated.[4–7] The studies revealed that the hydroxyethyl glucosides[4] **5** and **6** are full agonists of IP_3 with ≈tenfold reduced activity in comparison with IP_3 (**4**). On the other hand, the corresponding adenine-containing acyclophostin[5] **7** is a pH-dependent partial agonist and exhibits a binding affinity in the range of IP_3. These results indicate that the conformationally more flexible analogue **7** cannot counterbalance the loss of Ca^{2+}-releasing potency and that the orientation of the phosphate at the 2′-position is of crucial importance for optimal binding of **1**. This assumption is also endorsed by the observation that ribophostin[6] **8**, and the recently reported[7] furanophostin **9**, are ten times more potent than **5** and **6**. It may therefore be concluded that the high activity of adenophostin A (**1**) can be ascribed to an optimal spatial arrangement of the 2′-phosphate and/or an additional cooperative interaction of the adeninyl moiety with a region in the vicinity of the IP_3 binding site.[8] In order to study in detail the effect of the spatial orientation of the 2′-phosphate on the biological activity, it would be of interest to prepare a deadeninylated analogue of adenophostin A in which the 2′-phosphate is part of a constrained spiro[4.5]decane constellation.[9]

In this paper, we describe the synthesis of the diastereoisomeric spirophostins (3*R*)-**10** and (3*S*)-**11**. In addition, the biological activity of both analogues in terms of stereochemistry and conformational properties is assessed by molecular modeling.

Results and Discussion

The construction of the precursors of the target spiroketals (3*R*)-**10** and (3*S*)-**11** from the known[10] ethyl (2′*S*,3′*S*)-2,6-di-*O*-benzyl-3,4-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-β-D-glucopyranoside (**12**) is presented in Scheme 1. Hydrol-

[a] Prof. Dr. J. H. van Boom, Dr. M. de Kort, A. D. Regenbogen, Dr. A. R. P. M. Valentijn, Dr. G. A. van der Marel
Leiden Institute of Chemistry, Gorlaeus Laboratories Leiden University, P.O. Box 9502, 2300 RA Leiden (The Netherlands)
Fax: (+31) 71-527-4307
E-mail: j.boom@chem.leidenuniv.nl

[b] Dr. R. A. J. Challiss
Department of Cell Physiology & Pharmacology
Maurice Shock Medical Sciences Building
University of Leicester, University Road, Leicester LE1 9HN (UK)

[c] Y. Iwata, Dr. S. Miyamoto
Exploratory Chemistry Research Laboratories
Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku
Tokyo 140-8710 (Japan)

  0947-6539/00/0615-2696 $ 17.50+.50/0

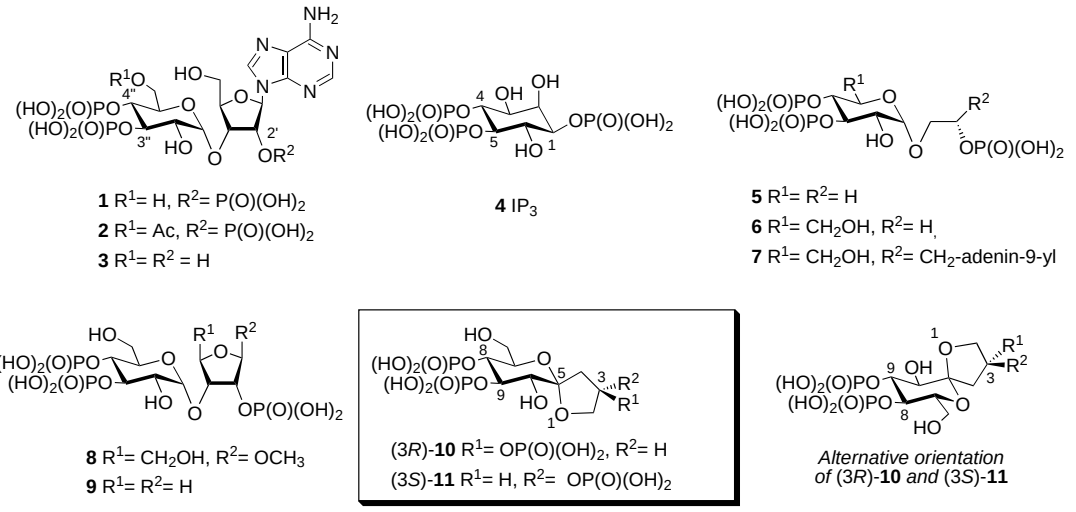
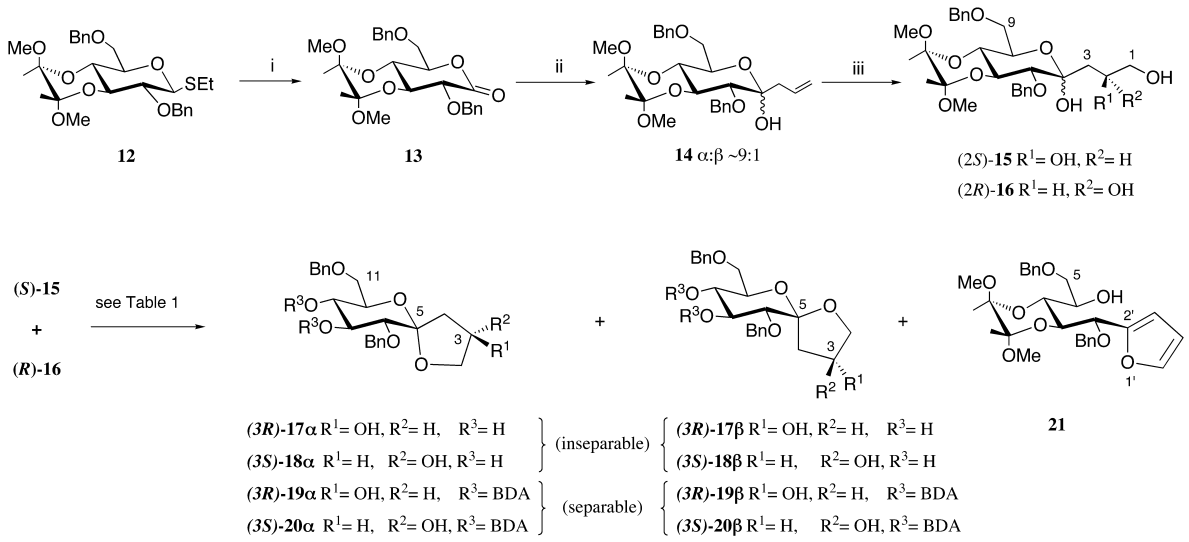


Figure 1. Structures of adenophostin A (**1**), analogues **3**–**9**, D-*myo*-inositol 1,4,5-trisphosphate IP_3 (**4**), and the spirophostins (3*R*)-**10** and (3*S*)-**11**.



Scheme 1. Reagents and conditions: i) 1. 0.1M NIS, TfOH CH_2Cl_2/THF/H_2O, 75:1:1, *v*/*v*/*v*, 88%; 2. DMSO/Ac_2O, 2:1, *v*/*v*, 2 h, 70°C, quant.; ii) Allylmagnesium bromide (1.05 equiv), THF, −78°C, 86% (α:β = 9:1); iii) cat. $K_2OsO_4 \cdot 2H_2O$, NMO (2.0 equiv), acetone/H_2O, 4:1, *v*/*v*, 1 h quant. (α:β = 9:1, (2*S*)-**15**:(2*R*)-**16** = 1:1).

ysis of **12** under the influence of *N*-iodosuccinimide (NIS) and subsequent oxidation of the anomeric hydroxy group under Albright–Goldman conditions[11] afforded lactone **13** in 88% yield over the two steps. Allylation of **13** with allylmagnesium bromide gave ketoglycoside **14** as a mixture of diastereoisomers (α:β, 9:1).[12] Treatment of the resulting olefin **14** with a catalytic amount of OsO_4 in the presence of *N*-methylmorpholine *N*-oxide led to an inseparable mixture of the protected nonulosides (2*S*)-**15** and (2*R*)-**16** (ratio = 1:1; α:β = 9:1) in a quantitative yield.[13]

At this stage, it was of interest to find out whether (2*S*)-**15** and (2*R*)-**16** could be converted under acidic conditions[14] into the suitably protected triols (3*R*)-**17*α*** and (3*S*)-**18*α***, required for the introduction of the phosphate groups. It can be seen (entry 1 in Table 1) that treatment of (2*S*)-**15** and (2*R*)-**16** with aqueous acetic acid led to spiroketalization and concomitant removal of the butane 3,4-diacetal (BDA) function, to give an intractable mixture of (3*R*)-**17*α*** and (3*S*)-**18*α***. Interestingly, subjection of (2*S*)-**15** and (2*R*)-**16** to a catalytic amount of camphorsulfonic acid (CSA) under anhydrous conditions in CH_2Cl_2 afforded the BDA-protected isomers (3*R*)-**19** and (3*S*)-**20** and the unexpected furan[15] derivative **21** in near equal amounts (entry 2, Table 1). Gratifyingly, execution of the same spiroketalization in MeOH as the solvent provided a separable mixture of the diastereoisomers (3*R*)-**19** and (3*S*)-**20** in good yield (entry 3, Table 1). In addition, the α-anomers of (3*R*)-**19** and (3*S*)-**20** were preferentially formed by executing the reaction at

Table 1. Spiroketalization of ketosugars (2*S*)-**15** and (2*R*)-**16**.

Entry	Conditions	**17** + **18** [%]	**19** + **20** [%]	α:β [a]	**21** [%]
1	HOAc/H_2O, 70/30, reflux, 2 h	63	0	9:1	0
2	CSA/CH_2Cl_2, 4 h	0	34	1:1	36
3	CSA/MeOH, 16 h	0	86	1:1	0
4	TfOH/MeOH, reflux, 6 h	0	60	19:1	0

[a] α = 5*R*; β = 5*S* ; all entries ratio 3*R*:3*S* = 1:1.

elevated temperature and using a more acidic catalyst (entry 4, Table 1). The identity of the individual spiroketals (3*R*)-**19*α*** and (3*S*)-**20*α*** was firmly established by mass spectrometry as well as ^{1}H NMR-NOESY spectroscopy. In addition, the configuration of HO-3 could be assigned unambiguously by spectroscopic analysis (Scheme 2) of the corresponding (*R*)- and (*S*)-Mosher ester (MTPA)[16] derivatives. Thus, the $\Delta\delta$ (ppm) values of the α-protons in the respective MTPA-derivatives were in full accordance with those predicted on the basis of the observed nuclear Overhauser effects.

Removal of the BDA protecting group (Scheme 3) in spiro[4.5]decanes (3*R*)-**19*α*** and (3*R*)-**19*β*** with aqueous trifluoroacetic acid (TFA) proceeded with concomitant isomerization of the spirocenter to give (3*R*)-**17*α*** as a single diastereoisomer. Similarly, conversion of the 3*S* isomers **20*α*** and **20*β*** led also to the exclusive formation of (3*S*)-**18*α***. The three hydroxy functions in **17** and **18** were phosphitylated with the monofunctional reagent dibenzyloxy-(*N,N*-diisopropylamino)phosphine[17] (**21**), followed by in situ oxidation of the resulting phosphite triesters with *tert*-butyl hydroperoxide, to yield the fully benzylated trisphosphates **22** and **23**. Purification of the debenzylated products by HW-40 gel filtration and Dowex-ion exchange chromatography gave the homogeneous spiroketals (3*R*)-**10** and (3*S*)-**11** (Na^+-salt) (Scheme 3), which were identified by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, as well as ES mass spectrometry.

Biological evaluation: The binding affinities of spirophostin (3*R*)-**10** and (3*S*)-**11** were compared with those of adenophostin A (**1**) and IP_3 (**4**) in ^{3}H-IP_3 displacement binding experiments using bovine adrenal cortex membranes.[18] The displacement curves and the estimated IC_{50} values of the four compounds are displayed in Figure 2 and Table 2, respective-

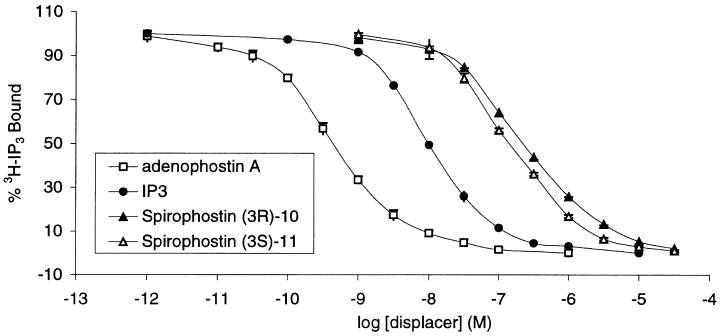
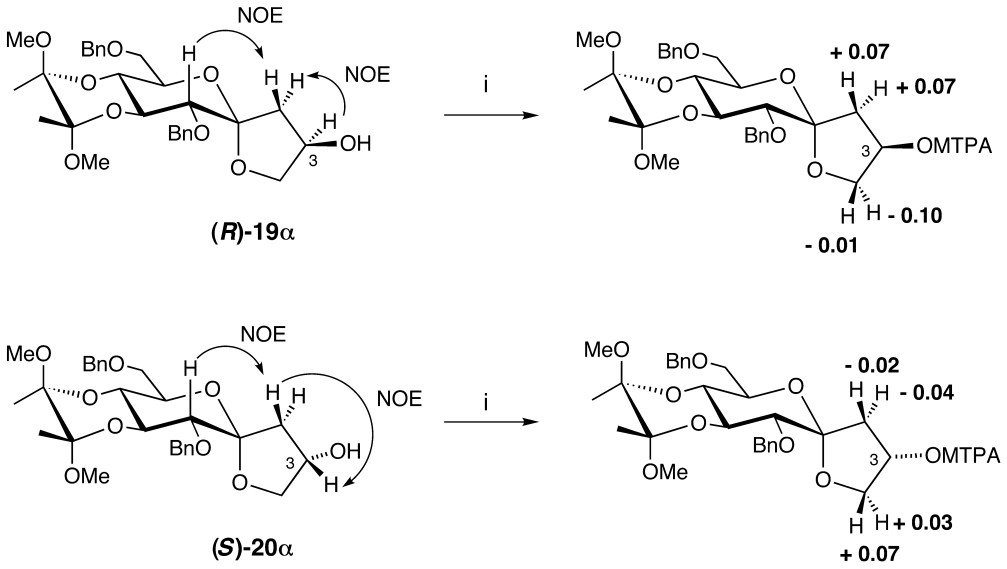
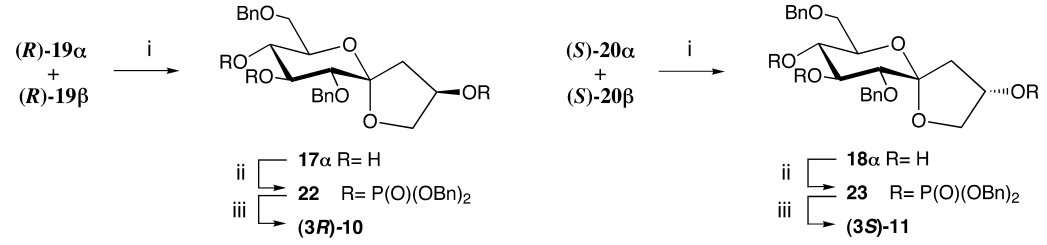


Figure 2. ^{3}H-IP_3 displacement isotherms for spirophostin (3*R*)-**10** and (3*S*)-**11** using bovine adrenal cortex membrane IP_3Rs. Data are expressed as percentage displacements of specific ^{3}H-IP_3 binding ($\pm$ s. e. mean for four experiments each performed in triplicate).



Scheme 2. Observed NOEs in spiroketals (3*R*)-**19*α*** and (3*S*)-**20*α*** and $\Delta\delta$ = values of the corresponding MTPA esters. Reagents and conditions: i) (*R*)-(−)- or (*S*)-(+)-2-methoxy-2-(trifluoromethyl)phenylacetic acid chloride (2.5 equiv), $(CH_2Cl)_2$/pyridine, mol. sieves (3 Å), 3 h; $\Delta\delta = \delta_R - \delta_S$ (ppm).



Scheme 3. Reagents and conditions: i) TFA/H_2O, 95:5, *v*/*v*, 2 h, 73% for **17*α***, 69% for **18*α***; ii) 1. **21**, 1*H*-tetrazole, $(CH_2Cl)_2$/CH_3CN, 3:1, *v*/*v*, 30 min; 2. *t*BuOOH, 0 °C, 30 min, 60% for **22**, 65% for **23**; iii) 10% Pd/C, H_2 (1 atm), NaOAc, 1,4-dioxane/propan-2-ol/H_2O, 4:2:1, *v*/*v*/*v*, 16 h, 87% for (3*R*)-**10**, 57% for (3*S*)-**11**.

0947-6539/00/0615-2698 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 15

Table 2. Spirophostin (3*R*)-**10** and (3*S*)-**11** ^{3}H-IP$_3$ displacement binding IC$_{50}$ values with respect to adenophostin A (**1**) and IP$_3$ (**4**).[a]

Entry	Compound	$-\log IC_{50}$	IC_{50} [nM]	*h*	n
1	adenophostin A (**1**)	9.409 ± 0.072	0.39	1.51 ± 0.13	3
2	IP$_3$ (**4**)	8.075 ± 0.062	8.4	0.99 ± 0.04	8
3	spirophostin (3*R*)-**10**	6.626 ± 0.022	237	0.82 ± 0.01	4
4	spirophostin (3*S*)-**11**	6.848 ± 0.012	142	0.90 ± 0.03	4

[a] Values are shown as ± s. e. mean for the concentration which causes 50% of specific ^{3}H-IP$_3$ displacement (IC$_{50}$), *h* the slope of the concentration-response curve, for *n* experiments.

ly. These data clearly show that the spirophostins are approximately 20-fold less effective than IP$_3$, while the relative displacing potencies of adenophostin A and IP$_3$ are consistent with earlier reported data. It is also evident (see Table 2) that the IC$_{50}$ for the *S* isomer **11** is roughly two times lower than for the *R* isomer **10**.

A functional response to the spirophostins was studied by measuring $^{45}Ca^{2+}$ release from intracellular stores upon binding to IP$_3$Rs in permeabilized SH-SY5Y neuroblastoma cells in comparison with the activities of adenophostin A (**1**) and IP$_3$ (**4**). The potency (Table 3) and slope (*h*) of the concentration-response curves (Figure 3) of (3*R*)-**10** and (3*S*)-**11** are again quite similar to each other in agreement with the

Table 3. Spirophostin (3*R*)-**10** and (3*S*)-**11** $^{45}Ca^{2+}$-release EC$_{50}$ values compared with those of adenophostin A (**1**) and IP$_3$ (**4**).[a]

Entry	Compound	$-\log EC_{50}$	EC_{50} [nM]	*h*	% Release	*n*
1	adenophostin A (**1**)	8.15 ± 0.04	7.03	1.51 ± 0.08	80.3 ± 5.4	4
2	IP$_3$ (**4**)	6.68 ± 0.09	208	0.99 ± 0.04	74.7 ± 6.9	4
3	spirophostin (3*R*)-**10**	5.88 ± 0.06	1306	1.10 ± 0.11	73.8 ± 4.3	4
4	spirophostin (3*S*)-**11**	5.59 ± 0.05	2594	1.02 ± 0.14	74.8 ± 3.6	4

[a] Values are shown as ± s. e. mean for the concentration which causes 50% of maximal $^{45}Ca^{2+}$ release (EC$_{50}$), with h as the slope of the concentration-response curve, the % release is relative to ionomycin-induced Ca^{2+} release, for *n* experiments.

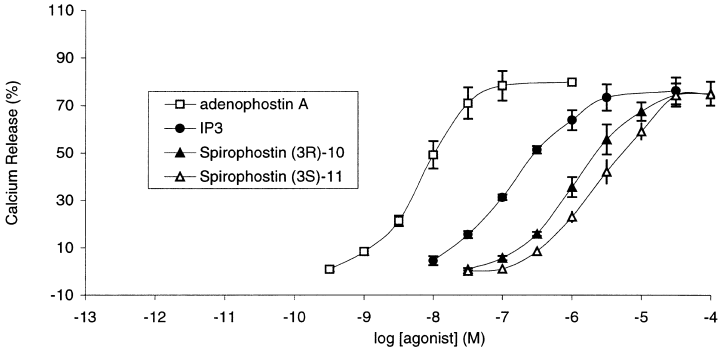


Figure 3. $^{45}Ca^{2+}$-Release response curves for spirophostin (3*R*)-**10** and (3*S*)-**11** using permeabilized SH-SY5Y neuroblastoma cells. Data are expressed as percentage $^{45}Ca^{2+}$ release (± s. e. mean for four experiments each performed in duplicate).

binding data presented in Table 2 (see entries 3 and 4). Interestingly however, the Ca^{2+}-releasing potency of spirophostin (3*R*)-**10** is higher than that of the corresponding isomer (3*S*)-**11**, and the potency order is opposite to that observed in the displacement experiments.

In summary, spirophostin (3*R*)-**10** and (3*S*)-**11** are approximately equipotent with respect to their IP$_3$R-binding and Ca^{2+}-releasing properties, and both are significantly less potent than IP$_3$ (**4**) and adenophostin A (**1**) with respect to their biological activity.

Molecular modeling: In order to rationalize the outcome of the biological experiments with respect to the three-dimensional structure of both spirophostins, a comparative molecular modeling study was undertaken. To this end, the energy-minimized conformers of (3*R*)-**10** and (3*S*)-**11**, as defined by Hotoda et al.,[3] were compared with the structures of IP$_3$ (**4**) and adenophostin A (**1**).

The conformational data as presented in Table 4 and Figure 4 reveal that the distance between the *trans*-8,9-

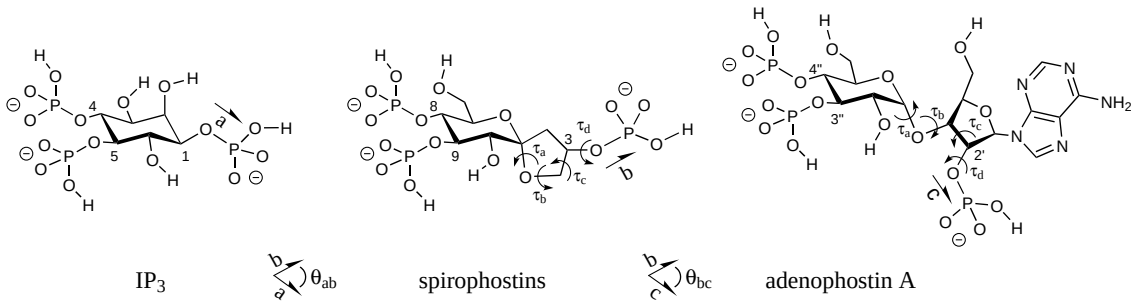


Figure 4. Torsion angles τ and directional angles θ_{ab} and θ_{bc} assigned in the molecular modeling.

Table 4. Conformational data for adenophostin A (**1**), IP$_3$ (**4**), spirophostin (3*R*)-**10** and (3*S*)-**11**.

Compound[a]	τ_a [°]	τ_b [°]	τ_c [°]	τ_d [°]	Distance between P atoms [Å] 1–4 (2′-4″)[b](3–8)[c]	1–5 (2′-3″)[b](3–9)[c]	4–5 (3″-4″)[b](8–9)[c]
IP$_3$ (**4**)	–	–	–	–	8.1	6.9	4.1
adenophostin A (**1**)	59	100	–23	–127	9.6	8.2	4.1
spirophostin (3*R*)-**10**	76	26	116	122	10.2	9.5	4.1
spirophostin (3*S*)-**11**	81	39	–147	–121	10.2	9.5	4.1

[a] Modeling of IP$_3$ and adenophostin A: see ref. [3]; for spirophostins the conformers *R*-**1** and *S*-**1** were used (see Experimental Section). [b] Numbering for adenophostin A. [c] Numbering for spirophostins.

bisphosphate moiety and the 3-phosphate (P-3) in both spirophostins is increased in comparison with adenophostin A and IP_3. Superimposition of (3*R*)-**10** with (3*S*)-**11** (see Figure 5A) shows that the interplay between different puckering of the five-membered ring in the spiroketals (i.e. *C*-5 *exo* in (3*R*)-**10** and *O*-1 *endo*/*C*-2 *exo* in (3*S*)-**11**) and an opposite value of the torsion angles τ_d results in nearly the same arrangement of the phosphorous atoms, in which P-3 is at a distance of 10.2 and 9.5 Å from P-8 and P-9, respectively (see Table 4).

Table 5. Distances [Å] and the directional angles θ between P-3, P-1, and P-2′ in superimpositioned models of (3*R*)-**10** and (3*S*)-**11** with IP_3 (**4**) and adenophostin A (**1**).

Compound	Superposition[a] 3–1	P–P distance [Å][b] 3–2′	θ_{ab} [°][c]	Direction of P-3 θ_{bc} [°][c]	
spirophostin (3*R*)-**10**	straight	2.7	1.8	61	36
	alternative	4.6	4.7	37	37
spirophostin (3*S*)-**11**	straight	2.8	2.0	30	37
	alternative	4.5	4.6	74	51

[a] Superimposition of six-membered rings, see Figure 5. [b] Left column: superimposition with IP_3; right column: superimposition with Adenophostin A. [c] See Figure 4.

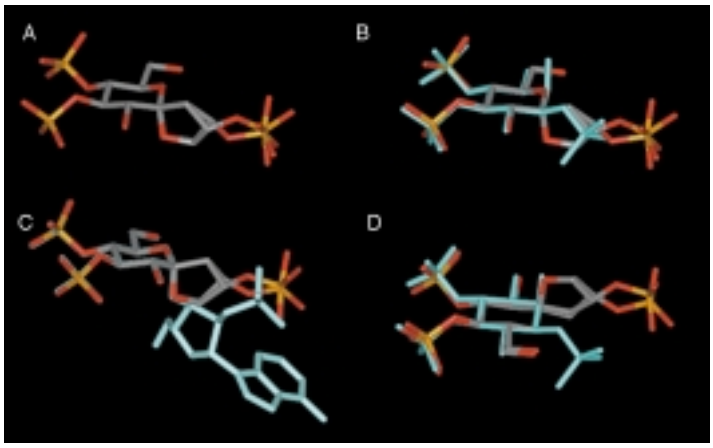


Figure 5. Superimposition of the six-membered rings in the energy-minimized structures. **A**. (3*R*)-**10** with (3*S*)-**11; B**. (3*R*)-**10** and (3*S*)-**11** with IP_3 (**4**, in blue); **C**. (3*R*)-**10** and (3*S*)-**11** with adenophostin A (**1**, in blue); **D**. Alternative orientation of (3*R*)-**10** and (3*S*)-**11** with IP_3 (**4**, in blue).

The relative location of P-3 in spirophostins (3*R*)-**10** and (3*S*)-**11** with respect to the corresponding phosphates P-1 and P-2′ in IP_3 and adenophostin A, respectively, was determined by comparison of the superimpositions of the respective energy-minimized conformers (Figure 5B and 5C). Perusal of Table 5 and Table 6 indicates that the distance between the P-3 in (3*R*)-**10** and the P-1 or P-2′ is slightly smaller than for the P-3 in (3*S*)-**11**. In this respect, it is of interest to note that a more distinct positioning of the P-3 does not occur owing to the high rotational energy barrier (>2 kcal mol^{-1}) of torsion angle τ_d. On the other hand, the direction of the P-3 in (3*S*)-**11**, as defined by the angle between the vectors along the O–P bonds (θ_{ab} and θ_{bc} in Figure 4 and Table 5), is more parallel to the direction of P-1 in IP_3 than in the case of (3*R*)-**10**.

It has been suggested[19] that the side of adenophostin A (**1**) which results from a 180° rotation around the C-3″–C-4″ bond in the glucose moiety would be responsible for binding to IP_3R. This alternative mode of binding is also not excluded for the spirophostins (see Figure 1), the more so, as the spiro-center mimics the axial HO-2 of IP_3 which tolerates bulky modifications.[20] However, alternative superimpositioning of (3*R*)-**10** and (3*S*)-**11** with the straight binding mode of IP_3 (Figure 5D) or adenophostin A reveals that in all cases the distance between P-3 and P-1 or P-2′ (see Table 5) is now approximately twofold increased, and indicates that this alternative mode of binding is highly unlikely.

In summary, based on the molecular modeling results it may be concluded that by virtue of the opposite geometry of their spiro[4.5]decane units, the phosphorous atoms at the C-3 stereogenic centers in (3*R*)-**10** and (3*S*)-**11** adopt similar orientations in space (see Figure 5A).

Conclusion

The conformationally restricted adenophostin A analogues (3*R*)-**10** and (3*S*)-**11** were successfully synthesized and evaluated for their biological activity. Molecular modeling showed

Table 6. P–P distances in most stable conformations of spirophostins (3*R*)-**10** and (3*S*)-**11**.

Stereo-Conf.No.	ΔE [kcal mol^{-1}]	Puckering of five-membered ring	P–P distances [Å] 3–8	3–9	8–9	Distance between 3-P and 2′-P[a] [Å] Model B	Model C
***R*-1**	**0.0**	**C5-*exo***	**10.2**	**9.5**	**4.1**	**1.8**	**3.3**
R-7	0.4	C5-*exo*/O1-*endo*	10.3	9.5	4.1	1.6	3.5
R-17	0.9	O1-*endo*/C5-*exo*	10.3	9.3	4.1	1.2	3.8
R-44	1.7	O1-*endo*	10.3	9.0	4.1	0.8	4.3
R-68	2.0	C2-*exo*-C3-*endo*	9.8	8.0	3.9	1.8	5.7
R-106	2.6	O1-*endo*/C2-exo	10.3	8.9	4.1	0.7	4.5
					Mean:	*1.3*	
***S*-1**	**0.0**	**O1-*endo*/C2-*exo***	**10.2**	**9.5**	**4.1**	**2.0**	**3.2**
S-7	0.3	C5-*exo*/O1-*endo*	9.5	9.6	4.1	3.4	2.3
S-9	0.5	O1-*endo*	10.0	9.7	4.1	2.5	2.8
S-12	0.7	O1-*endo*-C2-*exo*	10.3	9.6	4.1	1.7	3.4
S-26	1.2	C2-*exo*/O1-*endo*	10.3	9.5	4.1	1.6	3.6
S-123	3.0	C2-*exo*	10.4	9.6	3.9	1.4	4.0
					Mean:	*2.1*	

[a] Distance between 3-P of spirophostin and 2′-P of adenophostin (Model B & C) [3] when the glucose rings of both molecules were superimposed.

0947-6539/00/0615-2700 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 15

that the conformation of both spirophostins are quite similar which could explain the small difference in their IP_3R-binding and Ca^{2+}-releasing properties. Moreover, the orientation of P-3 in spirophostins (3*R*)-**10** and (3*S*)-**11** prevents optimal interaction with the receptor, thus explaining the reduced biological activities in comparison with adenophostin A (**1**) and IP_3 (**4**). The latter observation, together with other reports,[3, 6, 7] suggests that the activity of IP_3 cannot be exceeded with conformationally restricted adenophostin A analogues lacking the inextricable interplay between the P-2′ and the adenine moiety.

Experimental Section

General methods and materials: CH_2Cl_2 and toluene were dried by distillation from P_2O_5 (5 g L^{-1}) and stored over molecular sieves 4 Å (Acros). Et_3N was refluxed for 2 h in the presence of CaH_2 (5 g L^{-1}) and subsequently distilled. CH_3CN (Rathburn), $CHCl_3$, 1,4-dioxane, propan-2-ol, DMF, DMSO (Baker), acetone, and THF (Acros), p. a. grade, were stored over molecular sieves 4 Å. MeOH (HPLC-grade, Rathburn) was stored over molecular sieves 3 Å. Acetic acid and acetic anhydride (p. a., Baker) were used as received. Butane-2,3-dione, camphorsulfonic acid, trifluoromethanesulfonic acid, sodium hydride and 1*H*-tetrazole (Acros), Dowex 50WX4, *tert*-butyl hydroperoxide (80% in di-*tert*-butyl peroxide), (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-(trifluoromethyl)phenylacetic acid chloride (Fluka), allylmagnesium bromide (1.0 M in THF), *N*-iodosuccinimide and 10% Pd/C (Aldrich), benzyl bromide, and trifluoroacetic acid (Merck) were used as received. Di-*O*-benzyl-(*N*,*N*-diisopropyl) phosphoramidite (**21**) was prepared as described.[17]

All experiments were performed under anhydrous conditions at room temperature unless stated otherwise. Reactions were monitored by TLC analysis conducted at Schleicher and Schüll DC Fertigfolien (F 1500 LS 254). Compounds were visualized by UV light and by spraying with 20% sulfuric acid in EtOH or ammonium molybdate (25 g L^{-1}) and ceric ammonium sulfate (10 g L^{-1}) in 10% aqueous H_2SO_4, followed by charring at 140 °C. Column chromatography was performed on silica gel 60, 0.063–0.200 mm (Baker) or for crucial separations on silica gel 60, 0.040–0.063 mm (Merck). ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded with a JEOL JNM-FX-200 (200/50.1/80.7 MHz), a Bruker WM-300 (300/75.1/121.0 MHz) or a Bruker DMX-600 spectrometer (600/150/242.1 MHz). All spectra were recorded at 200/50.1/80.7 MHz, respectively, unless otherwise stated. ^{1}H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard and ^{31}P chemical shifts relative to 85% H_3PO_4 as external standard. Elemental analyses were performed with a Perkin–Elmer Series II Analyzer 2400. Mass spectra were recorded on a Finnigan MAT TSQ-70 or a PE-SCIEX API 165 mass spectrometer equipped with an Electrospray Interface (ES). Optical rotations were measured at 589 nm with an automatic Propol polarimeter.

(2′*S*,3′*S*)-2,6-Di-*O*-benzyl-3,4-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-D-glucono-1,5-lactone (13): To a vigorously stirred solution of ethyl (2′*S*,3′*S*)-2,6-di-*O*-benzyl-3,4-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-β-D-glucopyranoside (**12**)[10] (2.9 g, 5.6 mmol) in CH_2Cl_2/H_2O (35 mL, 10:1, *v*/*v*) was added a solution of *N*-iodosuccinimide (7.5 mmol) and TfOH (50 μL, 0.56 mmol) in CH_2Cl_2/THF (40:1, *v*/*v*) in a dropwise manner. After 2 h the reaction mixture was washed with aqueous $Na_2S_2O_3$ (20%), aqueous $NaHCO_3$ (10%), brine, and H_2O. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was subjected to column chromatography (Et_2O/light petroleum, 1:2, *v*/*v*) to afford the glucopyranose derivative. Yield 2.3 g, (4.9 mmol, 88%). R_f 0.34 (Et_2O/light petroleum, 2:1, *v*/*v*). The product (2.3 g, 4.88 mmol) was coevaporated with toluene (3 × 10 mL) and stirred in a mixture of DMSO (18 mL) and acetic anhydride (9 mL). After 2 h at 70 °C, when TLC analysis indicated the complete conversion into a higher-running product, the reaction mixture was concentrated and evaporated with toluene (3 × 10 mL). The crude product **13**, susceptible to hydrate formation, was used immediately in the next reaction without purification. R_f = 0.92 (Et_2O/light petroleum, 2:1, *v*/*v*); ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 168.2 (C-1), 137.1, 137.0 (2 × Cq Ph), 127.5, 127.4, 126.8 (CH arom), 98.4 (2 × Cq BDA), 76.8, 75.5, 68.5, 62.1 (C-2, C-3, C-4, C-5), 73.2, 72.4 (2 × CH_2 Bn), 47.0 (2 × CH_3 OMe), 16.8 (2 × CH_3 BDA).

(2′*S*,3′*S*)-5,9-Di-*O*-benzyl-6,7-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1,2,3-trideoxy-D-gluconon-1-en-4-ulopyranose (14): A solution of allylmagnesium bromide in THF (1.0 M, 4.55 mL) was added dropwise to a cooled (−78 °C) solution of compound **13** (2.1 g, 4.5 mmol) in THF (45 mL) under a N_2 atmosphere. After stirring for 30 min the mixture was quenched with aqueous NH_4Cl (10%), extracted with EtOAc, and washed with H_2O. The organic layer was dried ($MgSO_4$) and concentrated. Purification by column chromatography (Et_2O/light petroleum, 1:2, *v*/*v*) afforded allyl ketosugar **14** as a colorless oil. $\alpha/\beta \approx 9{:}1$. Yield 2.0 g, (3.9 mmol, 86%). R_f = 0.82 (EtOAc/light petroleum, 3:1, *v*/*v*); ^{1}H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ = 7.38–7.23 (m, 10 H, H arom), 5.92–5.78 (m, 1 H, H-2), 5.15 (dd, 1 H, H-1a, $J_{1a,1b}$ = 2.1 Hz, $J_{1a,2}$ = 10.2 Hz), 5.08 (dd, 1 H, H-1b, $J_{1b,2}$ = 17.2 Hz), 4.88 (AB, 2 H, CH_2 Bn, J = −11.2 Hz), 4.59 (AB, 2 H, CH_2 Bn, J = −12.2 Hz), 4.19 (t, 1 H, H-6, $J_{5,6} = J_{6,7}$ = 9.8 Hz), 4.08 (ddd, 1 H, H-8, $J_{7,8}$ = 10.2 Hz, $J_{8,9a}$ = 4.3 Hz, $J_{8,9b}$ = 2.0 Hz), 3.76 (t, 1 H, H-7), 3.73 (ABX, 2 H, H-9, $J_{9a,9b}$ = −11.3 Hz), 3.54 (d, 1 H, H-5, $J_{5,6}$ = 9.7 Hz), 3.31, 3.22 (2 × s, 6 H, 2 × CH_3 OMe), 2.91 (s, 1 H, OH), 2.47 (dd, 2 H, H-3, $J_{2,3}$ = 7.2 Hz, $J_{1b,3}$ = 0.8 Hz), 1.35, 1.30 (2 × s, 6 H, 2 × CH_3 BDA); ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 138.0 (2 × Cq Ph), 132.2 (C-2), 127.8–126.9 (CH arom), 118.8 (C-1), 99.0 (2 × Cq BDA), 97.8 (C-4), 77.5, 71.4, 69.7, 65.8 (C-5, C-6, C-7, C-8), 74.4, 72.8 (2 × CH_2 Bn), 67.8 (C-9), 47.5, 47.4 (2 × CH_3 OMe), 42.8 (C-3), 17.5, 17.3 (2 × CH_3 BDA); MS (ES): 532 [M+NH_4]$^+$, 537 [M+Na]$^+$, 553 [M+K]$^+$; $C_{29}H_{38}O_8$: calcd C 67.69, H 7.44; found: C 67.48, H 7.43%.

(3*R*) and (3*S*) (5*S*,7*R*,8*R*,9*S*,10*R*,2′*S*,3′*S*)-10-Benzyloxy-7-benzyloxymethyl-8,9-di-*O*-(2′,3′-dimethoxy-2′,3′-dioxybutane-2′,3′-diyl)-3-hydroxy-1,6-dioxaspiro[4.5]decane; (3R)-19α and (3*S*)-20α: To a solution of compound **14** (0.89 g, 1.7 mmol) in acetone/H_2O (20 mL, 4:1, *v*/*v*) was added *N*-morpholine oxide (0.50 g, 3.4 mmol), followed by a catalytic amount of $K_2OsO_4 \cdot 2H_2O$ (HAZARDOUS!, 31 mg, 0.08 mmol). After the mixture had been stirred for 1 h, TLC analysis revealed complete conversion of starting material into a lower running product. The mixture was stirred over 1 h in the presence of Na_2SO_3 (3 g) and extracted with Et_2O. The organic layer was washed with brine, H_2O, and dried over $MgSO_4$. Removal of the solvents yielded an inseparable mixture of triols (2*S*)-**15**+(2*R*)-**16** (ratio 1:1) in quantitative yield that was used without purification in the next reaction. R_f = 0.33 (EtOAc/light petroleum, 3:1, *v*/*v*); MS (ES): 571 [MNa]$^+$, 587 [M+K]$^+$. The crude mixture of triols (2*S*)-**15**+(2*R*)-**16** (0.34 g, 0.62 mmol) was treated according to entries 3 and 4 in Table 1. TLC analysis (light petroleum/EtOAc, 1:3, *v*/*v*) indicated the conversion of starting material into higher running products. In cases when CSA or TfOH was used the reaction mixture was neutralized with Et_3N prior to concentration under reduced pressure. Separation of the isomers was achieved by column chromatography (MeOH/EtOAc/toluene, 2.5:25:90, *v*/*v*/*v*; silica gel 0.040–0.063 mm) to give the two isomers in near equal amounts. The 3*R* and 3*S* isomers were separable as mixtures of α/β anomers. (3*R*)-**19α**: Yield 0.14 g (0.27 mmol, 44%); R_f = 0.61 (toluene/EtOAc/MeOH, 180:50:5, *v*/*v*/*v*); ^{1}H NMR (600 MHz, H–H COSY, $CDCl_3$): δ = 7.45–7.24 (m, 10 H, H arom), 4.87 (AB, 2 H, CH_2 Bn, J = −10.7 Hz), 4.56 (AB, 2 H, CH_2 Bn, J = −12.2 Hz), 4.23 (t, 1 H, H-9, $J_{8,9} = J_{9,10}$ = 9.9 Hz), 4.17 (ddd, 1 H, H-3, $J_{2a,3}$ = 2.1 Hz, $J_{3,4a}$ = 5.1 Hz, $J_{OH,3}$ = 11.6 Hz), 4.00 (ddd, 1 H, H-7, $J_{7,11b}$ = 1.9 Hz, $J_{7,11a}$ = 4.2 Hz, $J_{7,8}$ = 10.3 Hz), 3.85 (dd, 1 H, H-2a, $J_{2a,2b}$ = −9.5 Hz, $^4J_{2a,4b}$ = 1.4 Hz), 3.82 (dd, 1 H, H-2b, $J_{2b,3}$ = 2.2 Hz), 3.80 (t, 1 H, H-8), 3.66 (ABX, 2 H, H-11, $J_{11a,11b}$ = −11.2 Hz), 3.58 (d, 1 H, H-10), 3.52 (d, 1 H, OH), 3.34, 3.21 (2 × s, 6 H, OMe BDA), 2.23 (dd, 1 H, H-4a, $J_{4a,4b}$ = −14.5 Hz), 2.01 (d, 1 H, H-4b), 1.36, 1.29 (2 × s, 6 H, CH_3 BDA); ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 138.1, 136.9 (2 × Cq Ph), 128.9–126.8 (CH arom), 107.2 (C-5), 99.4, 99.3 (2 × Cq BDA), 77.8 (C-3), 75.8, 75.4 (2 × CH_2 Bn), 73.4 (C-2), 72.0, 71.9, 71.1, 66.0 (C-7, C-8, C-9, C-10), 68.0 (C-11), 48.0 (2 × CH_3 OMe), 46.1 (C-4), 17.8, 17.6 (2 × CH_3 BDA); $[\alpha]_D^{20}$ +123.2 (c = 1.0 $CHCl_3$); MS (ES): 548 [M+NH_4]$^+$, 553 [M+Na]$^+$; $C_{29}H_{38}O_9$: calcd C 65.64, H 7.22; found: C 65.38, H 7.19.

(3*S*)-**20α**: 0.14 g (0.26 mmol, 42%); R_f = 0.55 (toluene/EtOAc/MeOH, 180:50:5, *v*/*v*/v); ^{1}H NMR (600 MHz, H–H COSY, $CDCl_3$): δ = 7.36–7.25 (m, 10 H, H arom), 4.82 (AB, 2 H, CH_2 Bn, J = −11.6 Hz), 4.53 (AB, 2 H, CH_2 Bn, J = −12.2 Hz), 4.26 (m, 1 H, H-3), 4.21 (t, 1 H, H-9, $J_{9,10} = J_{8,9}$ = 9.8 Hz), 4.16 (dd, 1 H, H-2a, $J_{2a,2b}$ = −9.9 Hz, $J_{2a,3}$ = 4.7 Hz), 4.07 (m, 1 H, H-7), 3.95 (d, 1 H, H-2b), 3.68–3.56 (m, 4 H, H-8, H-11, H-10), 3.31, 3.21 (2 × s, 6 H, 2 × OMe BDA), 3.20 (d, 1 H, OH, $J_{OH,3}$ = 11.0 Hz), 2.16 (dd, 1 H,

H-4a, $J_{4a,4b} = -13.4$ Hz, $J_{3,4a} = 5.7$ Hz), 1.86 (d, 1H, H-4b), 1.36, 1.29 (2 × s, 6H, CH_3 BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): $\delta = 138.4$, 137.4 (2 × Cq Ph), 128.6–127.6 (CH arom), 109.5 (C-5), 99.7 (2 × Cq BDA), 79.2 (C-3), 75.4, 75.1, 75.0 (C-2, 2 × CH_2 Bn) 72.1, 71.9, 70.9, 66.2 (C-7, C-8, C-9, C-10), 66.2 (C-11), 48.1, 48.0 (2 × CH_3 OMe), 42.0 (C-4), 17.9, 17.7 (2 × CH_3 BDA); $[\alpha]_D^{20}$ +106.8 ($c = 1.0$ $CHCl_3$); MS (ES): 548 $[M+NH_4]^+$, 553 $[M+Na]^+$; $C_{29}H_{38}O_9$: calcd C 65.64, H 7.22; found: C 65.44, H 7.20.

(1*R*,2″*S*,3″*S*)-1,5-Di-*O*-benzyl-2,3-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-1-(2′-furyl)-D-lyxitol (21): Spiroketalization of triols (2*S*)-**15**+(2*R*)-**16** (0.25 g, 0.46 mmol) according to the conditions listed in entry 2 of Table 1 gave, in addition to the spiroketals (3*R*)-**19*α*** and (3*S*)-**20*α***, furan derivative **21** as a higher running product according to TLC analysis. Yield 36% (85 mg, 0.17 mmol); $R_f = 0.82$ (toluene/EtOAc/MeOH, 180:50:5, *v*/*v*/*v*); ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.41-7.16$ (m, 11H, H-5′, H arom), 6.50 (d, 1H, H-2′, $J_{2',3'} = 3.4$ Hz), 6.37 (dd, 1H, H-4′, $J_{3',4'} = 1.8$ Hz), 4.91 (d, 1H, H-1, $J_{1,2} = 2.9$ Hz), 4.53 (AB, 2H, CH_2 Bn, $J = -12.4$ Hz), 4.49 (s, 2H, CH_2 Bn), 4.18 (dd, 1H, H-3, $J_{3,4} = 5.7$ Hz, $J_{2,3} = 9.6$ Hz), 3.82 (dd, 1H, H-2, $J_{1,2} = 2.9$ Hz), 3.74–3.55 (m, 3H, H-4, H-5), 3.23 (s, 3H, CH_3 OMe), 3.21 (br s, 1H, OH), 2.91 (s, 3H, CH_3 OMe), 1.28, 1.22 (2 × s, 6H, 2 × CH_3 BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): $\delta = 152.2$ (C-2′), 141.5 (C-5′), 138.1, 137.9 (2 × Cq Ph), 128.6–127.5 (CH arom), 110.4, 109.1 (C-3′, C-4′), 99.0, 98.3 (2 × Cq BDA), 72.8, 71.7, 70.0, 68.3 (C-1, C-2, C-3, C-4), 73.1, 70.7, 70.2 (2 × CH_2 Bn, C-5), 47.8, 47.5 (2 × CH_3 OMe), 17.4, 16.9 (2 × CH_3 BDA); MS (ES): 535 $[M+Na]^+$, 551 $[M+K]^+$; $C_{29}H_{36}O_8$: calcd C 67.95, H 7.08; found: C 68.08, H 7.06.

Preparation of the Mosher ester derivatives of (3*R*)-19*α* and (3*S*)-20*α*: The BDA-protected spiroketal **19*α*** or **20*α*** (3.3 mg, 6.2 μmol) and (*N*,*N*-dimethyl)aminopyridine (0.2 mg, 1.6 μmol) were coevaporated with freshly distilled (CaH_2) pyridine (2 × 1 mL). The residue was dissolved in a mixture of 1,2-dichloroethane and pyridine (3 mL, 2:1, *v*/*v*) which was concentrated to a volume of 0.5 mL. The solution was stirred with molecular sieves (3 Å) under an Ar atmosphere and subsequently (*R*)-(−)- or (*S*)-(+)-2-methoxy-2-(trifluoromethyl)phenylacetic acid chloride (2.9 μL, 16 μmol) was added by syringe. After 3 h the reaction mixture was filtered over a layer of silica gel (0.6 × 6.0 cm) which was eluted with 1,2-dichloroethane (10 mL). The solution was washed with H_2O (5 mL), 2% aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), dried over $MgSO_4$ and concentrated. Traces of organic solvents were removed by coevaporation with carbon tetrachloride. The products obtained in this way were of enough purity for analysis by NMR spectroscopy. Note that the use of (*R*)-acid results in the formation of the (*S*)-ester. See Scheme 2 for a summary of the spectroscopic analysis of the Mosher esters.

***R*-Mosher ester derivative of (3*R*,5*S*,7*R*,8*R*,9*S*,10*R*,2′*S*,3′*S*)-10-benzyloxy-7-benzyloxymethyl-8,9-di-*O*-(2′,3′-dimethoxy-2′,3′-dioxybutane-2′,3′-diyl)-3-hydroxy-1,6-dioxaspiro[4.5]decane**: ^1H NMR (600 MHz, H–H COSY, $CDCl_3$): $\delta = 7.59-7.21$ (m, 15H, H arom), 5.50 (m, 1H, H-3), 4.76 (AB, 2H, CH_2 Bn, $J = -11.5$ Hz), 4.55 (AB, 2H, CH_2 Bn, $J = -12.1$ Hz), 4.19 (t, 1H, H-9, $J_{9,10} = J_{8,9} = 9.8$ Hz), 4.15 (dd, 1H, H-2a, $J_{2a,2b} = -10.7$ Hz, $J_{2a,3} = 5.9$ Hz), 4.05 (dd, 1H, H-2b, $J_{2b,3} = 2.2$ Hz), 3.84 (ddd, 1H, H-7, $J_{7,8} = 10.0$ Hz, $J_{7,11a} = 2.0$ Hz, $J_{9,11b} = 4.5$ Hz), 3.74 (t, 1H, H-8), 3.69–3.57 (m, 3H, H-11, H-10), 3.48 (s, 3H, OMe MTPA ester), 3.29, 3.19 (2 × s, 6H, 2 × OMe BDA), 2.30 (dd, 1H, H-4a, $J_{4a,4b} = -13.9$ Hz, $J_{3,4a} = 8.0$ Hz), 2.12 (dd, 1H, H-4b, $J_{3,4b} = 4.5$ Hz), 1.34, 1.28 (2 × s, 6H, 2 × CH_3 BDA); MS (ES): 769 $[M+Na]^+$.

***S*-Mosher ester derivative of (3*R*,5*S*,7*R*,8*R*,9*S*,10*R*,2′*S*,3′*S*)-10-benzyloxy-7-benzyloxymethyl-8,9-di-*O*-(2′,3′-dimethoxy-2′,3′-dioxybutane-2′,3′-diyl)-3-hydroxy-1,6-dioxaspiro[4.5]decane**: ^1H NMR (600 MHz, H–H COSY, $CDCl_3$): $\delta = 7.49-7.24$ (m, 15H, H arom), 5.52 (m, 1H, H-3), 4.82 (AB, 2H, CH_2 Bn, $J = -11.6$ Hz), 4.55 (AB, 2H, CH_2 Bn, $J = -12.3$ Hz), 4.19 (t, 1H, H-9, $J_{8,9} = J_{9,10} = 9.8$ Hz), 4.14 (dd, 1H, H-2a, $J_{2a,2b} = -10.6$ Hz, $J_{2a,3} = 6.2$ Hz), 3.95 (dd, 1H, H-2b, $J_{2b,3} = 2.3$ Hz), 3.84 (ddd, 1H, H-7, $J_{7,8} = 10.2$ Hz, $J_{7,11a} = 1.9$ Hz, $J_{7,11b} = 4.3$ Hz), 3.75 (t, 1H, H-8), 3.64 (ABX, 2H, H-11, $J_{11a,11b} = -11.0$ Hz), 3.58 (d, 1H, H-10), 3.42 (s, 3H, OMe MTPA ester), 3.29, 3.20 (2 × s, 6H, 2 × OMe BDA), 2.36 (dd, 1H, H-4a, $J_{4a,4b} = -13.8$ Hz, $J_{3,4a} = 8.0$ Hz), 2.19 (dd, 1H, H-4b, $J_{3,4b} = 4.9$ Hz), 1.34, 1.26 (2 × s, 6H, 2 × CH_3 BDA); MS (ES): 769 $[M+Na]^+$.

***R*-Mosher ester derivative of (3*S*,5*S*,7*R*,8*R*,9*S*,10*R*,2′*S*,3′*S*)-10-benzyloxy-7-benzyloxymethyl-8,9-di-*O*-(2′,3′-dimethoxy-2′,3′-dioxybutane-2′,3′-diyl)-3-hydroxy-1,6-dioxaspiro[4.5]decane**: ^1H NMR (600 MHz, H–H COSY, $CDCl_3$): $\delta = 7.51-7.23$ (m, 15H, H arom), 5.39 (m, 1H, H-3), 4.83 (AB, 2H, CH_2 Bn, $J = -11.5$ Hz), 4.43 (AB, 2H, CH_2 Bn, $J = -12.1$ Hz), 4.34 (dd, 1H, H-2a, $J_{2a,2b} = -10.3$ Hz, $J_{2a,3} = 6.1$ Hz), 4.18 (t, 1H, H-9, $J_{8,9} = J_{9,10} = 9.8$ Hz), 3.86 (dd, 1H, H-2b, $J_{2b,3} = 3.1$ Hz), 3.85 (t, 1H, H-8, $J_{7,8} = 9.9$ Hz), 3.81 (ddd, 1H, H-7, $J_{7,11a} = 1.8$ Hz, $J_{7,11b} = 3.0$ Hz), 3.66 (ABX, 2H, H-11, $J_{10a,10b} = -13.6$ Hz), 3.57 (d, 1H, H-10), 3.51 (s, 3H, OMe MTPA ester), 3.31, 3.21 (2 × s, 6H, 2 × OMe BDA), 2.41 (dd, 1H, H-4a, $J_{4a,4b} = -14.4$ Hz, $J_{3,4a} = 7.8$ Hz), 2.03 (dd, 1H, H-4b, $J_{3,4b} = 1.3$ Hz), 1.35, 1.28 (2 × s, 6H, 2 × CH_3 BDA); MS (ES): 769 $[M+Na]^+$.

***S*-Mosher ester derivative of (3*S*,5*R*,7*R*,8*R*,9*S*,10*R*,2′*S*,3′*S*)-10-benzyloxy-7-benzyloxymethyl-8,9-di-*O*-(2′,3′-dimethoxy-2′,3′-dioxybutane-2′,3′-diyl)-3-hydroxy-1,6-dioxaspiro[4.5]decane**: ^1H NMR (600 MHz, H–H COSY, $CDCl_3$): $\delta = 7.50-7.21$ (m, 15H, H arom), 5.36 (m, 1H, H-3), 4.88 (AB, 2H, CH_2 Bn, $J = -11.9$ Hz), 4.46 (AB, 2H, CH_2 Bn, $J = -12.2$ Hz), 4.37 (dd, 1H, H-2a, $J_{2a,2b} = -10.3$ Hz, $J_{2a,3} = 6.0$ Hz), 4.19 (t, 1H, H-9, $J_{8,9} = J_{9,10} = 9.6$ Hz), 3.93 (dd, 1H, H-2b, $J_{2b,3} = 3.7$ Hz), 3.86 (m, 2H, H-8, H-7), 3.68 (ABX, 2H, H-11, $J_{11a,11b} = -11.5$ Hz, $J_{7,11a} = 2.9$ Hz, $J_{7,11b} = 4.2$ Hz), 3.56 (d, 1H, H-10), 3.46 (s, 3H, OMe MTPA ester), 3.32, 3.21 (2 × s, 6H, 2 × OMe BDA), 2.40 (dd, 1H, H-4a, $J_{4a,4b} = -14.4$ Hz, $J_{3,4a} = 8.0$ Hz), 1.99 (d, 1H, H-4b), 1.35, 1.29 (2 × s, 6H, 2 × CH_3 BDA); MS (ES): 769 $[M+Na]^+$.

(3*R*,5*S*,7*R*,8*R*,9*S*,10*R*)-10-Benzyloxy-7-benzyloxymethyl-3,8,9-trihydroxy-1,6-dioxaspiro[4.5]decane [(3*R*)-17*α*]: The *α*/*β* mixture of spiroketal (3*R*)-**19** obtained in entry 3, Table 1 (0.19 g, 0.36 mmol) was dissolved in aqueous trifluoroacetic acid (95%, 8 mL) and was stirred for 2 h. The reaction mixture was concentrated in vacuo and coevaporated with toluene (2 × 5 mL). The oily residue was purified by column chromatography (light petroleum/EtOAc, 1:1 → 0:1, *v*/*v*) to give (3*R*)-**17*α*** as a white foam. Yield 73% (0.11 g, 0.26 mmol). ^1H NMR (600 MHz, H–H COSY, $CDCl_3$): $\delta = 7.44-7.25$ (m, 10H, H arom), 4.90 (AB, 2H, CH_2 Bn, $J = -11.0$ Hz), 4.56 (AB, 2H, CH_2 Bn, $J = -12.2$ Hz), 4.20 (m, 1H, H-3), 4.03 (t, 1H, H-9, $J_{8,9} = J_{9,10} = 9.3$ Hz), 3.85 (dd, 1H, H-2a, $J_{2a,2b} = -9.4$ Hz, $^4J_{2a,4b} = 1.4$ Hz), 3.83 (m, 1H, H-7), 3.80 (dd, 1H, H-2b, $J_{2b,3} = 2.5$ Hz), 3.64 (ABX, 2H, H-11, $J_{7,11a} = 4.3$ Hz, $J_{7,11b} = 4.2$ Hz, $J_{11a,11b} = -10.5$ Hz), 3.57 (t, 1H, H-8, $J_{7,8} = 9.6$ Hz), 3.40 (d, 1H, H-10), 3.38 (br s, 3H, OH), 2.16 (dd, 1H, H-4a, $J_{4a,4b} = -14.5$ Hz, $J_{3,4a} = 5.4$ Hz), 1.95 (dd, 1H, H-4b); $^{13}C\{^1H\}$ NMR ($CDCl_3$): $\delta = 136.8$ (2 × Cq Ph), 129.2–127.7 (CH arom), 107.1 (C-5), 79.5, 75.6, 72.5, 71.8, 71.5 (C-3, C-7, C-8, C-9, C-10), 75.7, 75.5 (2 × CH_2 Bn), 73.6 (C-2), 70.0 (C-11), 45.5 (C-4); MS (ES): 439 $[M+Na]^+$; $[\alpha]_D^{20}$: +8.8 ($c = 0.5$ $CHCl_3$); $C_{23}H_{28}O_7$: calcd C 66.33, H 6.78; found: C 66.39, H 6.77.

(3*S*,5*S*,7*R*,8*R*,9*S*,10*R*)-10-Benzyloxy-7-benzyloxymethyl-3,8,9-trihydroxy-1,6-dioxaspiro[4.5]decane [(3*S*)-18*α*]: The *α*/*β* mixture of spiroketal (3*S*)-**20** obtained in entry 3, Table 1 (99 mg, 0.19 mmol) was converted into (3*S*)-**18*α*** as described for the preparation of (3*R*)-**17*α***. Yield 69% (55 mg, 0.13 mmol); ^1H NMR (600 MHz, H–H COSY, $CDCl_3$): $\delta = 7.42-7.23$ (m, 10H, H arom), 4.77 (AB, 2H, CH_2 Bn, $J = -11.6$ Hz), 4.53 (AB, 2H, CH_2 Bn, $J = -12.1$ Hz), 4.28 (m, 1H, H-3), 4.13 (dd, 1H, H-2a, $J_{2a,2b} = -9.9$ Hz, $J_{2a,3} = 4.6$ Hz), 3.96 (d, 1H, H-2b), 3.94 (t, 1H, H-9, $J_{8,9} = J_{9,10} = 9.2$ Hz), 3.88 (m, 1H, H-7), 3.60 (ABX, 2H, H-11, $J_{7,11a} = 3.2$ Hz, $J_{7,11b} = 6.6$ Hz, $J_{11a,11b} = -10.3$ Hz), 3.44 (t, 1H, H-8, $J_{7,8} = 9.2$ Hz), 3.42 (d, 1H, H-10), 3.10 (br s, 3H, OH), 2.13 (dd, 1H, H-4a, $J_{4a,4b} = -13.4$ Hz, $J_{3,4a} = 5.7$ Hz), 1.87 (d, 1H, H-4b); $^{13}C\{^1H\}$ NMR ($CDCl_3$): $\delta = 137.7$, 137.6 (2 × Cq Ph), 128.5–127.6 (CH arom), 107.7 (C-5), 78.8, 75.2, 71.8, 71.3, 70.5 (C-3, C-7, C-8, C-9, C-10), 77.9, 75.2, 73.4, 69.9 (2 × CH_2 Bn, C-2, C-11), 41.5 (C-4); MS (ES): 439 $[M+Na]^+$; $[\alpha]_D^{20}$: +21.2 ($c = 0.5$ $CHCl_3$); $C_{23}H_{28}O_7$: calcd C 66.33, H 6.78; found: C 66.27, H 6.76.

(3*R*,5*S*,7*R*,8*R*,9*S*,10*R*)-10-Benzyloxy-7-benzyloxymethyl-3,8,9-trihydroxy-1,6-dioxaspiro[4.5]decane 3,8,9-tris-(di-*O*-benzyl)phosphate (22): A mixture of compound (3*R*)-**17*α*** (85 mg, 0.20 mmol) and dibenzyloxy-(*N*,*N*-diisopropylamino) phosphine[17] (**21**, 0.27 mL, 0.82 mmol) was dried by coevaporation with 1,4-dioxane (2 × 5 mL) and dissolved in 1,2-dichloroethane (6 mL). A solution of 1*H*-tetrazole (72 mg, 1.0 mmol) in acetonitrile (3.0 mL) was added under a N_2 atmosphere. After 30 min TLC analysis (light petroleum/Et_2O, 1:1, *v*/*v*) showed complete conversion of starting material into a higher running product ($R_f = 0.76$). The reaction mixture was cooled (0 °C), *tert*-butyl hydroperoxide (0.47 mL, 80% in di-*tert*-butyl peroxide) was added and stirring was continued for 30 min. TLC analysis revealed complete disappearance of the phosphite triester intermediate into a lower running product. The reaction mixture was diluted with EtOAc, washed with H_2O, and dried ($MgSO_4$), and concentrated in vacuo. Compound **22** was obtained as a colorless oil after purification by column chromatography (light petroleum/EtOAc, 3:1 → 0:1, *v*/*v*). Yield 60% (0.15 g, 0.12 mmol); R_f 0.53 (EtOAc/light petroleum, 3:2, *v*/*v*); ^1H NMR ($CDCl_3$, 300 MHz, H–H COSY): $\delta = 7.42-7.03$ (m, 40H, H arom), 5.11–

0947-6539/00/0615-2702 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 15

4.85 (m, 14H, 6 × CH_2 Bn, H-3, H-9), 4.71 (AB, 2H, CH_2 Bn, J = −11.5 Hz), 4.63 (q, 1H, H-8, $J_{8,9} = J_{7,8} = {}^3J_{8,p} = 9.6$ Hz), 4.41 (AB, 2H, CH_2 Bn, J = −12.1 Hz), 4.05 (dd, 1H, H-2a, $J_{2a,2b}$ = −10.2 Hz, ${}^3J_{2a,4} = 1.6$ Hz), 3.91–3.82 (m, 2H, H-2b, H-7), 3.71 (ABX, 2H, H-11, $J_{7,11a} = 4.4$ Hz, $J_{7,11b} = 1.8$ Hz, $J_{11a,11b} = -11.0$ Hz), 3.57 (d, 1H, H-10, $J_{9,10} = 9.7$ Hz), 2.17 (m, 2H, H-4); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 138.1, 137.5, 136.1–135.4 (Cq Bn), 133.0–127.5 (CH arom), 107.3 (C-5), 79.6, 77.9, 76.6, 74.6, 70.5 (C-3, C-7, C-8, C-9, C-10), 75.0, 73.0, 72.4 (2 × CH_2 Bn, C-2), 69.8–69.3 (CH_2 Bn), 68.1 (C-11), 41.3 (C-4); ^{31}P NMR ($CDCl_3$): δ = −0.67, −1.15, −1.57; MS (ES): 1198 $[M+H]^+$, 1220 $[M+Na]^+$; $[\alpha]_D^{20}$: +17.8 (c = 1.0 $CHCl_3$); $C_{65}H_{67}O_{16}P_3$: calcd C 65.21, H 5.64; found: C 65.25, H 5.63.

(3*S*,5*S*,7*R*,8*R*,9*S*,10*R*)-10-Benzyloxy-7-benzyloxymethyl-3,8,9-trihydroxy-1,6-dioxaspiro[4.5]decane 3,8,9-tris(di-*O*,*O*-benzyl)phosphate (23): Spiroketal (3*S*)-**18*α*** (55 mg, 0.13 mmol) was phosphorylated (as described for the preparation of **22**) to give **23**. Yield 65% (0.10 g, 85 μmol). R_f 0.64 (EtOAc/light petroleum, 3:2, *v*/*v*); ^1H NMR ($CDCl_3$): δ = 7.41–7.11 (m, 40H, H arom), 5.07–4.82 (m, 14H, 6 × CH_2 Bn, H-3, H-9), 4.63 (q, 1H, H-8, $J_{8,9} = J_{7,8} = {}^3J_{8,p} = 9.6$ Hz), 4.45 (AB, 2H, CH_2 Bn, J = −11.9 Hz), 4.36 (AB, 2H, CH_2 Bn, J = −12.4 Hz), 4.09 (dd, 1H, H-2a, $J_{2a,2b}$ = −9.9 Hz, $J_{2a,3} = 4.4$ Hz), 4.00 (m, 2H, H-7), 3.90 (d, 1H, H-2b), 3.72 (ABX, 2H, H-11, $J_{7,11a} = 4.3$ Hz, $J_{7,11b} = 1.9$ Hz, $J_{11a,11b} = -11.4$ Hz), 3.50 (d, 1H, H-10, $J_{9,10}$ = 9.8 Hz), 2.05 (m, 2H, H-4); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 138.2, 137.6, 135.7 (Cq Bn), 129.1–127.0 (CH arom), 106.9 (C-5), 79.9, 79.8, 76.8, 74.1, 69.9 (C-3, C-7, C-8, C-9, C-10), 75.2, 72.8, 70.7 (2 × CH_2 Bn, C-2), 69.9–69.4 (CH_2 Bn), 67.9 (C-11), 40.0 (C-4); ^{31}P NMR ($CDCl_3$): δ = −0.55 (P-3), −1.56 (P-8, P-9); MS (ES): 1220 $[M+Na]^+$; $[\alpha]_D^{20}$: 11.4 (c = 1.0 $CHCl_3$); $C_{65}H_{67}O_{16}P_3$: calcd C 65.21, H 5.64; found: C 65.15, H 5.58.

(3*R*,5*S*,7*R*,8*R*,9*S*,10*R*)-7-Hydroxymethyl-3,8,9,10-tetrahydroxy-1,6-dioxaspiro[4.5]decane 3,8,9-trisphosphate; spirophostin (3*R*)-10: Compound **22** (0.15 g, 0.12 mmol) was dissolved in a mixture of 1,4-dioxane, propan-2-ol and H_2O (15 mL, 4:2:1, *v*/*v*/*v*) containing NaOAc (0.12 g, 1.4 mmol). The clear solution was degassed and 10% Pd/C (75 mg) was added under an atmosphere of N_2. The mixture was stirred under H_2 (1 atm) for 16 h and the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure and the crude product was purified by gel-filtration over a Fractogel HW-40 column (elution: 0.15 M triethyl ammonium bicarbonate buffer). Concentration and coevaporation (MeOH/H_2O, 4:1, *v*/*v*, 3 × 5 mL) of the appropriate fractions, followed by lyophilization gave trisphosphate (3*R*)-**10** in pure form. The product was converted into the Na^+-form by ion-exchange with Dowex 50Wx4 (Na^+-form) followed by lyophilization. Yield 87% (64 mg, 0.11 mmol). ^1H NMR (D_2O, 600 MHz, H–H-COSY): δ = 4.87 (m, 1H, H-3), 4.28 (q, 1H, H-9, $J_{8,9} = J_{7,8} = {}^3J_{9,P} = 9.0$ Hz), 4.05 (ABX, 2H, H-2, $J_{2a,3} = 4.9$ Hz, $J_{2b,3} = 1.9$ Hz, $J_{2a,2b} = -10.1$ Hz), 3.96 (q, 1H, H-8, ${}^3J_{8,P} = 9.7$ Hz), 3.84 (dd, 1H, H-11a, $J_{11a,7} = 4.3$ Hz, $J_{11a,11b} = -13.5$ Hz), 3.66 (m, 3H, H-11b, H-7, H-10), 2.35 (d, 2H, H-4, $J_{3,4} = 5.5$ Hz); $^{13}C\{^1H\}$ NMR (D_2O): δ = 108.8 (C-5), 79.4 (C-3, $J_{3,P} = 3.1$ Hz), 74.8, 73.0, 72.0 (C-7, C-8, C-9, C-10, $J_{8,P} = 5.0$ Hz, $J_{9,P} = 5.3$ Hz), 74.3 (C-2, ${}^3J_{2,P} = 4.6$ Hz), 60.7 (C-11), 42.9 (C-4, ${}^3J_{4,P} = 5.0$ Hz); $^{31}P\{^1H\}$ NMR (D_2O, 242 MHz, P–H-COSY): δ = 2.83 (P-8), 2.35 (P-9), 1.68 (P-3); MS (ES): 475 $[M-H]^-$, 497 $[M-2H+Na^+]^-$, 519 $[M-3H+2Na^+]^-$; $[\alpha]_D^{20}$: +23.8 (c = 0.4 H_2O); HRMS (ES) calcd $C_9H_{18}O_{16}P_3$ $(M-H)^-$ 474.9807; found 474.9821.

(3*S*,5*S*,7*R*,8*R*,9*S*,10*R*)-7-Hydroxymethyl-3,8,9,10-tetrahydroxy-1,6-dioxaspiro[4.5]decane 3,8,9-trisphosphate; spirophostin (3*S*)-11: Deprotection of **23** (0.10 g, 85 μmol) and purification of the resulting trisphosphate was accomplished as described for the synthesis of spirophostin (3*R*)-**10** to afford the Na^+-salt of (3*S*)-**11**. Yield 57% (30 mg, 60 μmol). ^1H NMR (D_2O, 600 MHz, H–H-COSY, 7 °C): δ = 4.65 (m, 1H, H-3), 4.16 (m, 2H, H-9, H-2a), 3.88 (m, 2H, H-8, H-2b), 3.71 (ABX, 2H, H-11a, $J_{7,11a} = 4.0$ Hz, $J_{7,11b} = 1.7$ Hz, $J_{11a,11b} = -13.2$ Hz), 3.66 (t, 1H, H-7, $J_{7,8} = 9.8$ Hz), 3.53 (d, 1H, H-10, $J_{9,10} = 9.4$ Hz), 2.48 (dd, 1H, H-4a, $J_{4a,4b} = -14.6$ Hz, $J_{3,4a}$ = 8.1 Hz), 1.99 (dd, 1H, H-4b, $J_{3,4b} = 2.1$ Hz); $^{13}C\{^1H\}$ NMR (D_2O): δ = 108.6 (C-5), 79.3 (C-3, $J_{3,P} = 5.8$ Hz), 73.9, 73.2, 73.0, 72.7 (C-7, C-8, C-9, C-10, $J_{8,P} = 5.1$ Hz, $J_{9,P} = 4.6$ Hz), 75.3 (C-2, ${}^3J_{2,P} = 4.9$ Hz), 61.2 (C-11), 41.7 (C-4, ${}^3J_{4,P} = 4.7$ Hz); $^{31}P\{^1H\}$ NMR (D_2O, 242 MHz, P-H-COSY): δ = 3.45 (P-3), 2.92 (P-8), 2.76 (P-9); MS (ES): 475 $[M-H]^-$, 497 $[M-2H+Na^+]^-$, 519 $[M-3H+2Na^+]^-$; $[\alpha]_D^{20}$: +14.8 (c = 0.1 H_2O); HRMS (ES) calcd $C_9H_{18}O_{16}P_3$ $(M-H)^-$ 474.9807; found 474.9816.

^{3}H-IP$_3$ Displacement binding experiments: A P_2 fraction of bovine adrenal cortex was prepared as described previously.[21] Increasing concentrations of adenophostin A,[22] IP_3, and the spirophostins (3*R*)-**10** and (3*S*)-**11** were incubated with a constant amount of ^{3}H-IP_3 (approx. 9000 d.p.m. (disintegrations per minute) per assay; stock: 21 Ci mmol^{-1}; NEN) and adrenal cortex membranes; incubations were stopped after 30 min at 4 °C by rapid vacuum filtration.[18] Nonspecific binding was defined in the presence of 10 μM IP_3. Each displacement isotherm (see Figure 2) was used to obtain an estimate of the IC_{50} value (see Table 2) using GraphPad Prism and are given as $-\log IC_{50}$ values (± s.e. mean).

$^{45}Ca^{2+}$-Release experiments: Assays were performed using SH-SY5Y human neuroblastoma cells (passage 20–30) essentially as described previously[23] with certain modifications. Confluent monolayers of SH-SY5Y cells were washed and harvested using 10 mM HEPES, 0.9% NaCl, 0.02% EDTA, pH 7.4 and recovered by centrifugation (400 × *g*, 3 min). Cells were resuspended in an intracellular-like buffer (ICB: 20 mM HEPES, 13 mM KCl, 2.5 mM $MgCl_2$, 2 mM ATP, 20 μM $CaCl_2$, Ph 7.1; the free $[Ca^{2+}]$ was buffered to 100–150 nM by addition of EGTA) and centrifuged (400 × *g*, 3 min). This latter step was repeated and the final cell pellet was gently resuspended in ICB supplemented with an ATP regenerating system (10 mM phosphocreatine, 10 U mL^{-1} creatine phosphokinase) and permeabilization was achieved by addition of 50 μg mL^{-1} β-escin. After 2 min 1 μCi mL^{-1} $^{45}Ca^{2+}$ (1000 Ci mmol^{-1}, Amersham, Little Chalfont, UK) was added and the permeabilized cell suspension was added to ICB containing different concentrations of adenophostin A (**1**), IP_3 (**4**), spirophostin (3*R*)-**10** or (3*S*)-**11**. Incubations were continued for 2 min (IP_3, 30 s) and samples were then centrifuged (13,000 × *g*, 3 min). A silicone oil mixture (300 μL of Dow-Corning 556/550, 1:1, *v*/*v*) was then added to each tube and the samples were recentrifuged (13 000 × *g*, 3 min). The ICB and oil were then aspirated, tubes inverted and allowed to drain for ≥ 60 min before addition of 1.1 mL FloScint IV scintillation cocktail (Packard Bioscience BV, Groningen, the Netherlands). Samples were stored overnight in the dark before scintillation counting. The total releaseable $^{45}Ca^{2+}$-pool was defined as that released by addition of 10 μM ionomycin. Each release isotherm was used to obtain estimates of the EC_{50} value, the slope factor (h) and the maximum obtainable release (expressed as a percentage of the total ionomycin-releaseable pool) using GraphPad Prism.

Molecular modeling: All calculations were run on IRIS workstations according to Hotoda et al.[3] The stable conformers (Table 6), within 3.0 kcal mol^{-1} from the lowest-energy conformers *R*-1 and *S*-1, were selected from a total of 434 and 363 stable conformations found for the *R* and *S* isomer, respectively. After determination of the distance between the P-3 of (3*R*)-**10** or (3*S*)-**11** and the P-2′ in superimpositions with two models of adenophostin A (models B and C in ref.[3]), it was established that the spirophostins matched better with model B. This model, which also corresponded better with the solution structure of adenophostin A assigned by NMR spectroscopy, was therefore used in Tables 4 and 5 in the Discussion.

Acknowledgement

We thank Rajendra Mistry (Department of Cell Physiology & Pharmacology, University of Leicester) for his excellent technical contribution to the IP_3R binding experiments and Cees Erkelens and Fons Lefeber for recording NMR spectra. We thank Hans van der Elst and Nico Meeuwenoord for recording mass spectra. This research has been financially supported by the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO).

[1] a) M. J. Takahashi, T. Kagasaki, T. Hosoya, S. Takahashi, *J. Antibiot.* **1993**, *46*, 1643–1647; b) S. Takahashi, T. Kinoshita, M. Takahashi, *J. Antibiot.* **1994**, *47*, 95–100; c) M. Takahashi, K. Tanzawa, S. Takahashi, *J. Biol. Chem.* **1994**, *269*, 369–372; d) J. Hirota, T. Michikawa, A. Miyawaki, M. Takahashi, K. Tanzawa, I. Okura, K. Mikoshiba, *FEBS Lett.* **1995**, *368*, 248–252; e) Y. Sato, S. Miyazaki, T. Shikano, N. Mitsuhashi, T. Hioyuki, K. Mikoshiba, Y. Kuwabara, *Biol. Reprod.* **1998**, *58*, 867–73.

[2] R. A. Wilcox, W. U. Primrose, S. R. Nahorski, R. A. J. Challiss, *Trends Pharmacol. Sci.* **1998**, *19*, 467–475.

[3] H. Hotoda, K. Murayama, S. Miyamoto, Y. Iwata, M. Takahashi, Y. Kawase, K. Tanzawa, M. Kaneko, *Biochemistry* **1999**, *38*, 9234–9241.

[4] a) D. J. Jenkins, B. V. L. Potter, *Carbohydr. Res.* **1996**, *287*, 169 – 182; b) R. A. Wilcox, C. Erneux, W. U. Primrose, R. Gigg, S. R. Nahorski, *Mol. Pharmacol.* **1995**, *47*, 1204 – 1211; c) N. Moitessier, F. Chrétien, Y. Chapleur, *Tetrahedron Lett.* **1995**, *44*, 8023 – 8026.
[5] a) N. C. R. van Straten, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1997**, *53*, 6523 – 6538; b) M. D. Beecroft, J. S. Marchant, A. M. Riley, N. C. R. van Straten, G. A. van der Marel, J. H. van Boom, B. V. L. Potter, C. W. Taylor, *Mol. Pharmacol.* **1999**, *1*, 109 – 117.
[6] a) D. J. Jenkins, R. D. Marwood, B. V. L. Potter, *Chem. Commun.* **1997**, 449; b) J. S. Marchant, M. D. Beecroft, A. M. Riley, D. J. Jenkins, R. D. Marwood, C. W. Taylor, B. V. L. Potter, *Biochemistry* **1997**, *36*, 12 780—12 790.
[7] a) S. Shuto, K. Tatani, Y. Ueno, A. Matsuda, *J. Org. Chem.* **1998**, *63*, 8815 – 8824; b) R. D. Marwood, A. M. Riley, V. Correa, C. W. Taylor, B. V. L. Potter, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 453 – 458.
[8] P.-J. Lu, D.-M. Gou, W.-R. Shieh, C.-S. Chen, *Biochemistry* **1994**, *33*, 11 586—11 597.
[9] In a similar approach, the synthesis of two D-*myo*-inositol-based bicyclic analogues was recently reported, however, a biological evaluation was not included: A. M. Riley, B. V. L. Potter, *Tetrahedron Lett.* **1999**, *40*, 2213 – 2216.
[10] M. de Kort, A. R. P. M. Valentijn, G. A. van der Marel, J. H. van Boom, *Tetrahedron Lett.* **1997**, *38*, 7629 – 7632.
[11] J. D. Albright, L. Goldman, *J. Am. Chem. Soc.* **1967**, *89*, 2416 – 2419.
[12] M. D. Lewis, J. K. Cha, Y. Kishi, *J. Am. Chem. Soc.* **1982**, *104*, 4976 – 4978.
[13] Attempts to improve the diastereoselectivity of the dihydroxylation using Sharpless' conditions were unsatisfactory, which in close agreement with similar experiments on other allylated hexoses. See: a) H. C. Kolb, M. S. VanNieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483 – 2547; b) M. K. Gurjar, A. S. Mainkar, *Tetrahedron: Asymmetry* **1992**, *3*, 21 – 24; c) N. Moitessier, F. Chrétien, Y. Chapleur, *Tetrahedron: Asymmetry* **1997**, *8*, 2889 – 2892, and ref. [16a].
[14] F. Perron, K. F. Albizati, *Chem. Rev.* **1989**, *89*, 1617 – 1673.
[15] R. Shiraki, A. Sumino, K. Tadano, S. Ogawa, *J. Org. Chem.* **1996**, *61*, 2845 – 2852.
[16] a) M. Sasaki, A. Hasegawa, K. Tachibana, *Tetrahedron Lett.* **1993**, *34*, 8489 – 8492; b) I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092 – 4096.
[17] a) W. Bannwarth, A. Trzeciak, *Helv. Chim. Acta* **1987**, *70*, 175 – 186; b) E. Dreef, G. A. van der Marel, J. H. van Boom, *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 161 – 164.
[18] R. A. J. Challiss, E. R. Chilvers, A. L. Willcocks, S. R. Nahorski, *Biochem. J.* **1990**, *265*, 421 – 427.
[19] C. T. Murphy, A. M. Riley, C. J. Lindley, D. J. Jenkins, J. Westwick, B. V. L. Potter, *Mol. Pharmacol.* **1997**, *52*, 741 – 748, and ref. [2] and [9].
[20] M. Hirata, F. Yanaga, T. Koga, T. Ogasawara, Y. Watanabe, S. Ozaki, *J. Biol. Chem.* **1990**, *265*, 8404 – 8408.
[21] R. A. J. Challiss, I. H. Batty, S. R. Nahorski, *Biochem. Res. Commun.* **1988**, *157*, 684 – 691.
[22] N. C. R. van Straten, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1997**, *53*, 6509 – 6522.
[23] R. A. Wilcox, R. A. J. Challiss, G. Baudin, A. Vasella, B. V. L. Potter, S. R. Nahorski, *Biochem. J.* **1993**, *294*, 191 – 194.

Received: December 29, 1999 [F2218]

  0947-6539/00/0615-2704 $ 17.50+.50/0