Synthesis of New Cyclitol Compounds That Influence the Activity of Phosphatidylinositol 4-Kinase Isoform, PI4K230

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The synthesis, chemical derivatization, and investigation of the inhibitory properties of novel cyclitol derivatives on the phosphatidylinositol 4-kinase enzymes PI4K55 and PI4K230 involved in the phosphatidylinositol cycle are reported. Some of the prepared cyclitol derivatives (i.e. **9**, **11**, **12**, and **14**) proved to be very powerful and specific irreversible inhibitors of PI4K230 at or below a concentration of 1 mM.

Introduction

Phosphatidylinositol (PtdIns) 4-kinase (PI4K) (EC 2.7.1.67) catalyzes the formation of phosphatidylinositol 4-phosphate (PtdIns-4P), which serves as the starting material for the biosynthesis of several regulatory phosphoinositides, being further phosphorylated at the D-5 and/or D-3 position of the cyclitol ring.[1,2] The diversity of physiological functions of PI4Ks located at several subcellular compartments can involve different phosphoinositide pools and might explain[3,4] the existence of the multiple forms of PI4K. According to their enzymological and immunological properties, domain structures, and molecular masses, the mammalian PI4Ks are grouped[4] into forms of PI4K55, PI4K92, and PI4K230. It is supposed that the plasma membrane-bound PI4K55 is involved in the agonist-stimulated activation of the phosphatidylinositol pathway, while other forms, reported to be associated[5−7] with intracellular membranes, can participate in the regulation of cytoskeletal actin-modifying proteins[8] and vesicular transport.[9]

PI4Ks are the targets of and possess high sensitivity to several microbial products, such as echiguanine[10] and wortmannin, which is a fungal sterol-like toxin.[5−7,11,12] Previously we have demonstrated[13] the inhibitory effect of (2*S*,3*R*,5*R*)-3-azido-2-benzoyloxy-5-hydroxycyclohexanone (**1**) on the enzyme D-*myo*-inositol-1-phosphate phosphatase of *Neurospora crassa*. Supposing structural similarities in the substrate-recognizing domain of the inositol (Ins) and PtdIns-metabolizing enzymes, we decided to test the effect of **1** as well as its derivatives and structural analogues on the activity of the PI4K enzymes (PI4K55 and PI4K230) involved in the phosphatidylinositol cycle.

Results and Discussion

Chemistry. In recent papers we have described the preparation[14] of novel azido, anhydro, and branched-chain chiral cyclitols from carbohydrates and from D-(−)-quinic acid, as well as their utilization for the production of pseudodisaccharide-type aminocyclitol antibiotics.[15] To synthesize cyclitol analogues carrying an exocyclic C=C bond suitable for further derivatization, the deoxyinososes **1**,[16] **2**,[17] and **3**[14] were treated with methoxycarbonylmethylenetriphenylphosphorane to obtain the methoxycarbonylmethylenecyclohexanes **4**−**6,** as single geometric isomers (TLC, NMR), in medium to good yield (Scheme 1). The structure of compounds (*E*)-**4**−**6** was deduced from the ^{1}H and ^{13}C NMR spectra, which showed all of the signals (see Tables 1 and 2) characteristic of the structural elements of the exocyclic =CH−COOCH$_3$ function. The *E* geometry for each of the three products was concluded from the large ($\Delta\delta$ = 0.9−1.1 ppm) downfield shift of the *axially* oriented H-6 proton as compared to that of the starting ketones **1**−**3**, indicating that the methoxycarbonylmethylene side chain is close to this proton. Inosose **7**[18] was transformed into the corresponding branched-chain cyclitol **8** (55%) in an essentially similar fashion. The NMR data (Tables 1 and 2) substantiated the development of the side chain, but the doubled signals clearly indicated the presence of two geometrical isomers in a ca. 3:2 ratio, which could not be separated by chromatography.

To obtain substituted and modified derivatives of the inososes **1**−**3** for structure−activity relationship (SAR) studies, the corresponding 5-*O*-acetyl derivatives **9**−**11** and the unsaturated ketones **12**, **13**,[14] **14**, and **15**[19] were prepared. To avoid β-elimination of the C-5 hydroxyl group of the deoxyinososes **1**−**3**, the acetylations were carried out with the acetic−trifluoroacetic mixed anhydride in dichloromethane. For the preparation of the α,β-unsaturated ketones **12**−**15**, the desired β-elimination reaction was facilitated by means of the introduction and elimination of an 5-*O*-methanesulfonyl leaving group. Incorporation of the double bond into the molecules of the resulting α,β-unsaturated ketones was shown by the appearance of carbon signals at δ = 148−195 ppm and of proton signals in the region δ = 6.10−7.20 ppm characteristic of related structures.[14]

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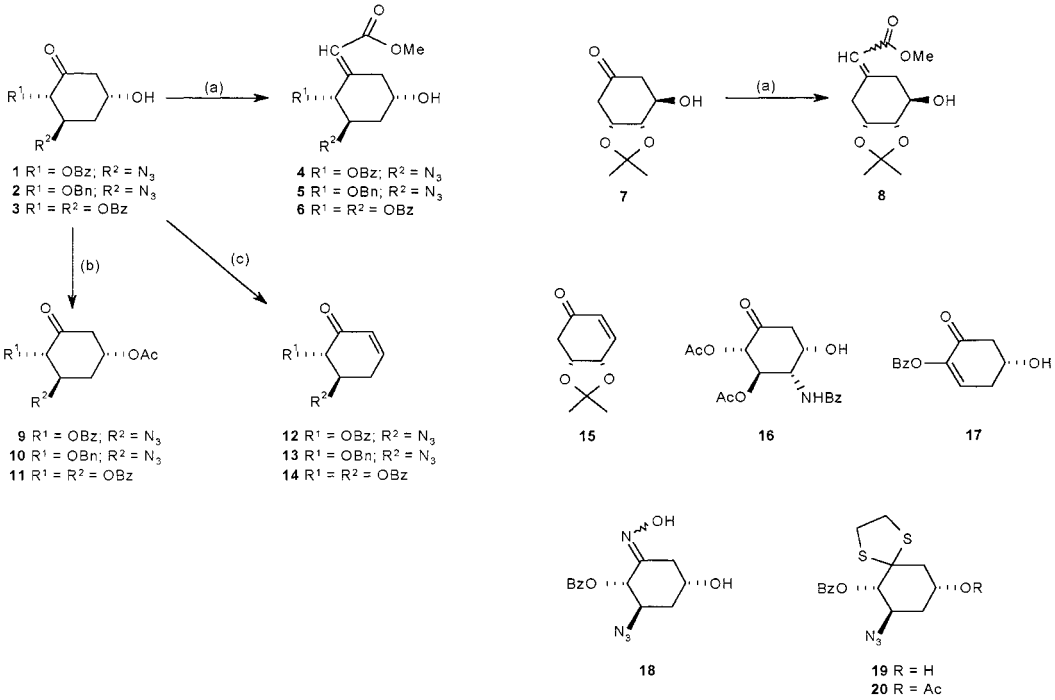
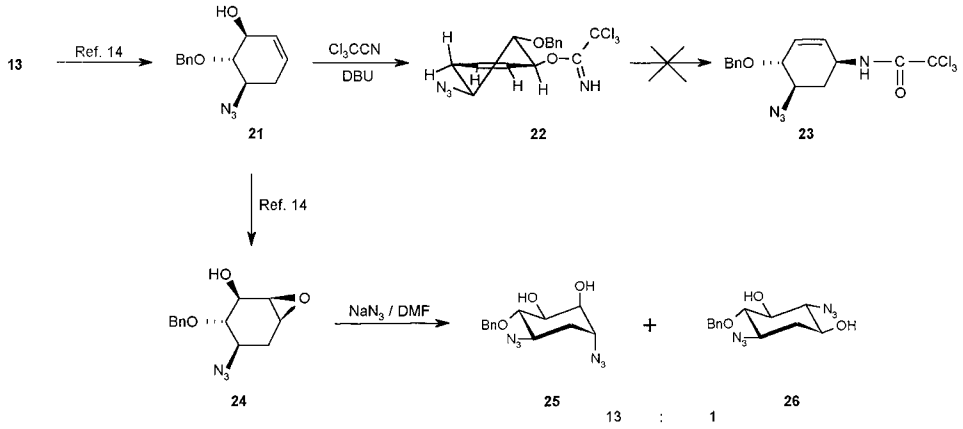
Scheme 1[a]



[a] (a) $Ph_3P{=}CHCOOCH_3$/toluene; (b) $(CF_3CO)_2O/CH_3COOH$; (c) CH_3SO_2Cl/pyr.

Scheme 2



To extend the scale of the samples for the SAR investigations, the aminodeoxyinososе **16**, derived[20] from D-glucosamine, the unsaturated ketone **17**,[17] and the 3-azidodeoxyinoseses **18**[17] and **19**,[15] carrying the carbonyl group in protected form and prepared earlier in our laboratory, were employed. The 5-*O*-acetylated derivative (**20**) of the dithioketal **19** was obtained by means of conventional acetylation of **19** with acetic anhydride in pyridine.

With the goal of synthesizing diazidocyclitol and diaminocyclitol derivatives, the latter being structurally related to the neuraminidase enzyme inhibitory aminocyclitols GS 4071 and GS 4104,[21] the well-known Overman rearrangement[22] of an appropriate trichloroacetimidate ester, such as the (1*S*,5*R*,6*S*)-allyl alcohol **21**,[14] was attempted (Scheme 2). Treatment of **21** with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane gave 97% of the target trichloroacetimidate ester **22**. The incorporation of the ester moiety was clearly proved by the ^1H and ^{13}C NMR spectral data: the proton of the imine function appeared at $\delta = 8.46$ ppm, and the carbons of the CCl_3 and the $C{=}NH$ blocks of the ester moiety were assigned at $\delta =$ 137.71 and 161.89 ppm, respectively. Thermal Overman rearrangement[22] of **22** in boiling xylene, or that performed at room temperature in dichloromethane under mercury(II) trifluoroacetate catalysis, did not give the desired unsaturated aminocyclitol **23**. Instead, a very small portion of the starting ester **22** was recovered, and extensive ester hydrolysis of the labile **22** to lead to **21** proceeded under the employed experimental conditions. The failure of the desired rearrangement may be attributed to the lability of the imidate ester **22** and the fact that it does not adopt a $_5T^6$ twist-boat conformation required[14] for an easy steric approach of the ester side chain to the β-position. In contrast, the thermal Claisen rearrangement of the allyl alcohol **21** (possessing an undistorted $_5T^6$ twist-boat conformation) with *N*,*N*-dimethylacetamide dimethyl acetal readily provided[14] the corresponding branched-chain cyclitol derivative, carrying a dimethylacetamide side chain, with a com-

Table 1. 200-MHz ^{1}H NMR Data for Compounds **4**−**6**, **8**−**14**, **20**, **22**, **25**, and **26**

		chemical shifts (δ, ppm)[a]								
compd	solvent	H-1	H-2	H-3	H-4	H-5	H-6	=C*H*−	O*H*	others
4	$CDCl_3$		5.49 (9.5)	4.09 (11.33, 4.75)	1.78(*a*) 2.33(*e*)	4.35 (6.58, 3.29)	2.33(*e*) 3.97(*a*)	5.99	2.73	−OC*H*$_3$ 3.69
5	acetone-d_6		4.07	3.90	1.77(*a*) 2.13(*e*)	4.10	2.62(*e*) 3.52(*a*)	6.15	2.82	−OC*H*$_2$Ph 4.67 −OC*H*$_3$ 3.67
6	$CDCl_3$		5.81 (8.77)	5.67 (9.14, 4.4)	2.04(*a*) 2.51(*e*)	4.41 (4.4)	2.64(*e*) 3.88(*a*)	6.10	2.57	−OC*H*$_3$ 3.69
8	$CDCl_3$		2.22−3.15	4.47	4.16	4.04	2.22−3.15	5.78	3.02	−C(C*H*$_3$)$_2$ 1.33, 1.45 −OC*H*$_3$ 3.58
	$CDCl_3$		2.72−3.15	4.49	4.10	3.94	2.22−3.15	5.76	3.02	−C(C*H*$_3$)$_2$ 1.33, 1.47 −OC*H*$_3$ 3.70
9	acetone-d_6		5.56	4.40	2.18(*a*) 2.42(*e*)	4.70	2.56 2.95			−COC*H*$_3$ 2.10
10	acetone-d_6		4.18	4.02	1.94(*a*) 2.12(*e*)	4.38	2.46(*a*) 2.78(*e*)			−COC*H*$_3$ 2.05
11	$CDCl_3$		5.78	5.94	2.20(*a*) 2.61(*e*)	4.58	2.85			−COC*H*$_3$ 2.06
12	acetone-d_6		6.14 (10.0)	7.15 (6.0, 2.5)	2.78(*a*) 3.05(*e*)	4.52 (12.0, 5.5)	5.68 (12.0)			
13	acetone-d_6		6.02	7.00	2.44(*a*) 2.88(*e*)	4.50	4.49			−OC*H*$_2$Ph 4.72, 5.08
14	acetone-d_6		6.21 (9.87)	7.20 (2.56, 5.85)	3.01(*a*) 3.24(*e*)	5.81 (9.50, 5.85, 11.70)	6.01 (11.70)			
20	acetone-d_6		5.39 (9.13)	4.16 (10.92, 4.34)	2.22 1.98	5.12	2.63			−S(C*H*$_2$)$_2$S− 3.18, 3.34 −COC*H*$_3$ 2.08
22	$CDCl_3$	5.68 (7.0)	5.76	5.76	2.14(*a*) 2.54(*e*)	3.80 (10.5, 6.0)	3.69 (10.5)			−OC*H*$_2$Ph 4.87 −C=N*H* 8.46
25	acetone-d_6	3.92 (3.5)	4.02 (8.0)	3.80 (9.0)	3.61	1.90−2.07 (3.5)	3.64 (2.5)		4.28, 4.51	−OC*H*$_2$Ph 4.86
26	acetone-d_6	3.60 (9.5, 9.5)	4.20	3.78	3.54−3.62	1.92 1.98	3.54−3.62		4.41, 4.48	−OC*H*$_2$Ph 4.79

[a] Coupling constants (in parentheses) are given in Hz.

Table 2. 200-MHz ^{13}C NMR Data for Compounds **4**−**6**, **8**, **14**, **22**, **25**, and **26**

		chemical shifts (δ, ppm)							
compd	solvent	C-1	C-2	C-3	C-4	C-5	C-6	=*C*H−	others
4	$CDCl_3$	151.42	77.14	61.62	35.20	66.13	36.75	115.86	−OCH$_3$ 51.50 −*C*=O 165.08, 167.04
5	acetone-d_6	155.31	84.10	63.40	35.54	66.57	37.39	116.98	−OCH$_3$ 51.25 −OCH$_2$Ph 73.01 −*C*=O 167.15
6	$CDCl_3$	151.21	75.64	72.43	35.38	66.63	36.71	117.28	−OCH$_3$ 51.67 −*C*=O 165.50, 165.62, 167.07
8	$CDCl_3$	155.27	31.90	72.20	76.51	67.65	35.35	116.62	−OCH$_3$ 50.79 −C(*C*H$_3$)$_2$ 24.22, 26.62
	$CDCl_3$	155.08	29.47	71.91	76.65	68.35	36.13	117.10	−OCH$_3$ 50.80 −C(*C*H$_3$)$_2$ 24.38, 26.89
12	acetone-d_6	170.24	148.87	134.57	32.50	61.05	79.26		
14	acetone-d_6	192.14	148.08	134.26	32.11	71.17	77.59		
20	acetone-d_6	67.05	80.02	60.58	34.38	68.83	44.14		−COCH$_3$ 21.34 −*C*OCH$_3$ 165.58 −S(*C*H$_2$)$_2$S− 39.34, 41.77
22	$CDCl_3$	80.93	128.36	124.18	31.11	60.26	79.90		−OCH$_2$Ph 74.79; −*C*=NH 161.89 −*C*Cl$_3$ 137.71
25	acetone-d_6	82.25	72.39	71.69	60.54	30.38	60.55		−OCH$_2$Ph 75.11
26	acetone-d_6	67.94	78.49	70.36	57.99	30.21	67.93		−OCH$_2$Ph 73.37

plete chirality transfer from carbon C-1 of the enol to the β-position.

As an alternative route for synthesizing diazido (and thus diamino) cyclitols, (1*R*,2*S*,3*R*,5*S*,6*R*)-3-azido-2-benzyloxy-5,6-anhydrocyclohexan-1-ol (**24**) prepared[14] earlier in our laboratory was applied. We have reported[14] that treatment of **24** with allyl alcohol in the presence of boron trifluoride etherate resulted in a *diaxial* opening of the oxirane ring with excellent regiospecificity and yield.

When the anhydro ring of **24** was opened with sodium azide in hot *N*,*N*-dimethylformamide, a ca. 13:1 mixture of the 4,6-diazido- (**25**) and 1,4-diazidocyclitol (**26**) with 1*S*,2*S*,3*S*,4*R*,6*R* and 1*R*,2*S*,3*S*,4*R*,6*S* configuration, respectively, was obtained and the pure isomers could be isolated by means of column chromatography. The large values of the $J_{2,3}$ and $J_{3,4}$ coupling constants (8 and 9 Hz, respectively) indicated the $^3C_6$ chair conformation of the major product **25**, which carries the newly introduced 1-OH and 6-N_3 groups in a *trans-diaxial* relationship ($J_{1,2} = 3.5$ Hz, $J_{1,6} = 2.5$ Hz, and $J_{5,6} = 3.5$ Hz). The minor product **26** also exists in the $^3C_6$ chair conformation, and this compound is a result of the *trans-diequatorial* ring opening ($J_{1,2} = J_{1,6} = 9.5$ Hz) of the oxirane function of **24** with sodium azide.

Biology. The cyclitol derivatives influenced the activity of the enzyme PI4K230 very differently. Some compounds, such as **9** and **11**, proved to be powerful inhibitors at a concentration of 1 mM exerting a clear-cut time-dependent inhibition (Figure 1). Other sub-

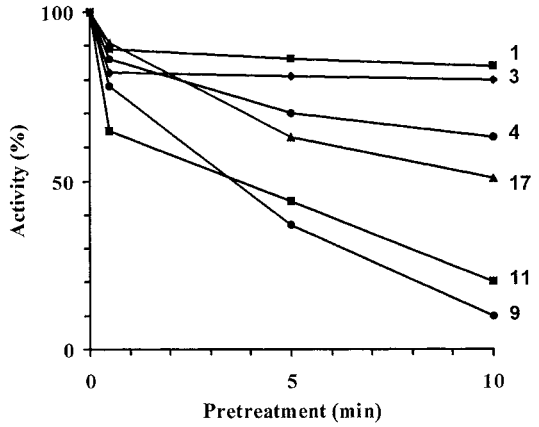


Figure 1. Time dependency of the inhibiton of PI4K230 with the cyclitol derivatives. The PI4K230 preparation was incubated with 1 mM cyclitol derivatives at 25 °C. At the indicated time points the samples were supplemented with the substrate, and the remaining activities were determined as described in the Experimental Section. The activities are expressed as a percent (%) of the appropriate controls run without the cyclitol derivatives. The compounds are numbered as shown in the schemes.

Table 3. Inactivation of the Enzyme PI4K230 by 19 Selected Cyclitol Derivatives[a]

entry	compd	remaining activity (%)	entry	compd	remaining activity (%)
1	**19**	96	11	**6**	64
2	**20**	96	12	**4**	63
3	**2**	85	13	**15**	57
4	**13**	85	14	**16**	56
5	**3**	84	15	**17**	51
6	**10**	82	16	**11**	20
7	**1**	80	17	**9**	10
8	**7**	80	18	**14**	8
9	**5**	70	19	**12**	5
10	**18**	66			

[a] The PI4K230 preparation was incubated with 1 mM of the compounds for 10 min at 25 °C, and the remaining activity was determined immediately as described in the Experimental Section.

stances (e.g. **1** and **3**) caused only about 20% inhibiton without any consistent time dependency. A third group possessed about 40−50% largely time-dependent inhibition such as **4** and **17**. Finally, some compounds were ineffecive within a 5% limit. The inhibition exerted by the compounds showed a concentration dependence, as well (not documented). To compare the efficiency of various derivatives the inactivation was performed regularly for 10 min.

By comparing the structure and the inhibitory effect of 19 selected cyclitols (Table 3) the following conclusions can be drawn. (a) The presence of a keto functional group, or an other group attached with a double bond to the cyclohexane ring, is a prerequirement for inhibition (see entries 3−18). Thus, the inososes **19** and **20** carrying the C-1 keto functions in a protected, dithioketal form are completely ineffective (entries 1 and 2). (b) An ester group (benzoyl or acetyl) attached to the 2-OH function can greatly enhance the inhibitory power, while the inosose derivatives carrying an ether substituent (e.g. a benzyl group) at the same position are not so effective. This is most significantly demonstrated by the huge differences between the inhibitory potencies of **12** and **13** (entries 19 and 4) or **9** and **10** (entries 17 and 6). (c) The substituents at carbons C-3 and C-4 do not appear to be critical in the inhibition of the enzyme PI4K230 (compare e.g. the data in entries 5, 7; 11, 12; 16, 17; and 18, 19). (d) Acetylation of the 5-OH hydroxyl group (see compounds **9** and **11** in entries 16 and 17) or elimination of the C-5 substituent, resulting in the cyclitols **14** and **12** with a conjugated unsaturated system (entries 18 and 19), furnished the inosose derivatives possessing the highest inhibitory activity.

Conclusion

The prepared cyclitol compounds influenced the activity of the phosphatidylinositol 4-kinase enzyme PI4K230 very differently. The observed time-dependent inhibition suggests an irreversible chemical modification of the enzyme decreasing its catalytic activity. The most powerful inhibitors contain a C-1 oxo, a C-2 benzoyloxy, and a C-5 acetoxy group or an unsaturated (cyclohexene) ring, suggesting the role of the multiple conjugated unsaturated system providing a close to planar structure for the inhibitory compounds. Quite interestingly, another type of the PI4K enzymes (such as PI4K55) do not show sensitivity to the cyclitol compounds which inactivated PI4K230. According to this finding, the inhibitory effect of the reported new cyclitol derivatives seems to be rather specific for PI4K230.

The structural basis of this difference is not clear since the amino acid sequence of PI4K55 is not yet known. However, several lipid kinases show structural similarity to PI4K230, such as PI4K92, type II β-phosphatidylinositol phosphate kinase, and phosphoinositide 3-kinases.[23,24] Among them PI4K92, PI4K230, and especially phosphoinositide 3-kinases proved to be sensitive to inhibition by wortmannin, while PI4K55 is not sensitive to this microbial toxin.[3,25]

Supposing similarities in the accessibility to wortmannin and cyclitol derivatives, our compounds might efficiently influence hormonal and mitogenic signaling mediated by phosphoinositide 3-kinases[25] or the functions of PI4K92 exerted in Golgi complex,[26] as well. It is noteworthy that PI4K230 seems to be involved predominantly in the vesicular traffic of neuronal cells;[27,28] however, its exact role has not yet been clarified.

Experimental Section

Chemistry. General. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 283 B instrument. ^{1}H (200 MHz) and ^{13}C NMR spectra (50.3 MHz) were recorded with a Bruker 200 SY spectrometer (internal TMS). Mass spectra were recorded with AEI-MS 902 and VG-7035 instruments. TLC and column chromatography were performed on Kieselgel 60 F_{254} (Merck) and silica gel 60 (Merck), using (A) 1:1 hexanes−ethyl acetate, (B) 6:4 hexanes−ethyl acetate, (C) 7:3 hexanes−ethyl acetate, (D) 9:1 dichloromethane−methanol, (E) 9:1 toluene−methanol, (F) 10:0.1 dichloromethane−methanol, (G) 8:2 hexanes−ethyl acetate, (H) 3:7 hexanes−ethyl acetate as the developing system/eluent. Evaporations were carried out under diminished pressure at 35−40 °C (bath temperature).

(2*S*,3*R*,5*R*)-3-Azido-2-benzoyloxy-5-hydroxy-1-(methoxycarbonylmethylene)cyclohexane (4). A mixture of the azidodeoxyinosose **1**[16] (102.5 mg, 0.37 mmol) and methoxycarbonylmethylenetriphenylphosphorane (150 mg, 0.44 mmol) in dry toluene (3.0 mL) was stirred at 70 °C for 23 h, when TLC (A) showed that all of the starting **1** had disappeared. The reaction mixture was evaporated to dryness and the residue

purified by flash-column chromatography (B) to obtain pure crystalline **4** (99.7 mg, 81.3%): mp 91−93 °C; $[\alpha]_D^{20} = -127$ (c = 0.77 $CHCl_3$); R_f 0.36 (A). Anal. ($C_{16}H_{17}N_3O_5$) C, H, N.

(2*S*,3*R*,5*R*)-3-Azido-2-benzyloxy-5-hydroxy-1-(methoxycarbonylmethylene)cyclohexane (5). To a stirred solution of **2**[15] (96.1 mg, 0.37 mmol) in dry toluene (3.5 mL) methoxycarbonylmethylenetriphenylphosphorane (156.1 mg, 0.47 mmol) was added, the mixture was refluxed for 2 h, and then stirred at 70 °C for 22 h. Following evaporation, the residue was purified by column chromatography (A→C) to obtain 53.0 mg (45.3%) of pure crystalline **5**: mp 68−69 °C; $[\alpha]_D^{20} = -154$ (c = 1.0 $CHCl_3$); R_f 0.72 (A), 0.24 (C). Anal. ($C_{16}H_{19}N_3O_4$) C, H, N.

(2*S*,3*R*,5*R*)-2,3-Dibenzoyloxy-5-hydroxy-1-(methoxycarbonylmethylene)cyclohexane (6). A mixture of **3**[17] (100 mg, 0.28 mmol) and methoxycarbonylmethylenetriphenylphosphorane (114 mg, 0.34 mmol) in dry toluene (2.0 mL) was stirred at 50 °C for 16 h, when TLC (A) showed that all of the starting material had disappeared. The reaction mixture was concentrated and the residue was submitted to flash-column chromatography to furnish pure crystalline **6** (100 mg, 87.2%): mp 127−129 °C; $[\alpha]_D^{20} = -91$ (c = 0.74 $CHCl_3$); R_f 0.44 (A). Anal. ($C_{23}H_{22}O_7$) C, H, N.

(3*R*,4*S*,5*R*)-3,4-Isopropylidenedioxy-5-hydroxy-1-(methoxycarbonylmethylene)cyclohexane (8). A mixture of **7**[18] (434 mg, 2.33 mmol) and methoxycarbonylmethylenetriphenylphosphorane (935 mg, 4.79 mmol) in dry toluene (10.0 mL) was stirred at 100 °C until all of the starting material had disappeared [19 h, TLC (D)]. The reaction mixture was then concentrated, and the residue was submitted to flash-column chromatography (D) to obtain pale yellow syrupy **8** (310 mg, 55%), as a 3:2 mixture of two isomers: R_f 0.62 (D). Anal. ($C_{12}H_{18}O_5$) C, H, N.

(2*S*,3*R*,5*R*)-3-Azido-2-benzoyloxy-5-acetoxycyclohexane (9). To a cold (0 °C) solution of trifluoroacetic anhydride (3.2 mL, 23.0 mmol) and glacial acetic acid (0.6 mL, 10.48 mmol) the deoxyinosose **1**[16] (400 mg, 1.45 mmol) was added and the reaction mixture was stirred for 16 h at room temperature. It was then poured into dilute aqueous $NaHCO_3$ solution and extracted with chloroform (2 × 15 mL). The organic layer was washed with water (2 × 10 mL), dried (Na_2SO_4) and concentrated. The residual syrupy product was purified by means of flash-column chromatography (E) to afford pure **9** (300 mg 65%): mp 115−117 °C. Anal. ($C_{15}H_{15}N_3O_5$) C, H, N.

(2*S*,3*R*,5*R*)-3-Azido-2-benzyloxy-5-acetoxycyclohexane (10). Acetylation of **2**[15] (366 mg, 1.20 mmol) with the mixed anhydride generated from trifluoroacetic anhydride (3.2 mL, 23.0 mmol) and glacial acetic acid (0.6 mL, 10.08 mmol) was carried out as described above for **9** to obtain, after column chromatography, amorphous **10**. Anal. ($C_{15}H_{17}N_3O_4$) H, N; C: calcd, 59.35; found, 58.33.

(2*S*,3*R*,5*R*)-2,3-Dibenzoyloxy-5-acetoxycyclohexane (11). Acetylation of **3**[17] (105 mg, 0.29 mmol) with the mixed anhydride generated from trifluoroacetic anhydride (0.6 mL, 4.31 mmol) and glacial acetic acid (0.12 mL, 2.09 mmol) was carried out as described above for **9** to give, after column chromatography, crystalline **11**: mp 128−130 °C. Anal. ($C_{22}H_{20}O_7$) H; C: calcd, 66.60; found, 65.33.

(5*R*,6*S*)-5-Azido-6-benzoyloxy-2-cyclohexen-1-one (12). To a stirred, cold (0 °C) solution of **1**[16] (0.25 g, 0.908 mmol) in dry pyridine (10 mL) was added dropwise methanesulfonyl chloride (0.10 mL, 1.20 mmol) and then the reaction mixture was allowed to warm to room temperature. After 2 h, it was diluted with dichloromethane (10 mL) and washed with saturated aqueous $NaHCO_3$ solution (2 × 10 mL) and water (2 × 10 mL), dried (Na_2SO_4) and concentrated. Coevaporation with dry toluene (2 × 15 mL) resulted in crude syrupy **12**, which was submitted to column chromatography (C) to furnish pure syrupy **12** (0.185 g, 79%): $[\alpha]_D^{20} = -124.4$ (c = 3.3 $CHCl_3$); R_f 0.6 (C); IR λ_{max} (KBr) 2106 (N_3), 1694 (C═O) cm^{-1}. Anal. ($C_{13}H_{11}N_3O_3$) C, H, N.

(5*R*,6*S*)-5,6-Dibenzoyloxy-2-cyclohexen-1-one (14). To a stirred, cold (0 °C) solution of **3**[17] (300 mg, 0.85 mmol) in dry pyridine (15 mL) was added dropwise methanesulfonyl chloride (0.1 mL) and then the reaction mixture was allowed to warm to room temperature. After 4−5 h, it was diluted with dichloromethane (30 mL) and washed with saturated aqueous $NaHCO_3$ solution (2 × 15 mL) and water (2 × 15 mL), dried ($MgSO_4$) and concentrated. Coevaporation with dry toluene (2 × 15 mL), followed by column chromatography (dichloromethane) resulted in pure crystalline **14** (279 mg, 98%): mp 106−108 °C; $[\alpha]_D^{20} = -146.5$ (c = 1.08 $CHCl_3$); R_f 0.76 (F). Anal. ($C_{20}H_{16}O_5$) C, H.

(2*S*,3*R*,5*R*)-3-Azido-5-acetoxy-2-benzoyloxycyclohexanone-ethylenedithioacetal (20). To a cold solution of **19**[15] (697 mg, 0.2 mmol) in dry pyridine (5.0 mL) acetic anhydride (21 mL, 0.22 mmol) was added, and the solution was stirred at room temperature for 24 h. It was then diluted with dichloromethane (15 mL), washed with saturated aqueous $NaHCO_3$ solution (2 × 10 mL) and water (2 × 5 mL), dried ($MgSO_4$) and concentrated to afford pure syrupy **20** (52.3 mg, 68%): mp 120−121 °C. Anal. ($C_{17}H_{19}N_3O_4S_2$) C, H, N, S.

(1*S*,5*R*,6*S*)-5-Azido-6-benzyloxy-1-(trichloroacetimidoyloxy)cyclohex-2-ene (22). To a stirred solution of **21**[14] (590 mg, 2.40 mmol) in dry dichloromethane (25 mL) trichloroacetonitrile (2.41 mL, 24.04 mmol) and DBU (179 mL, 1.202 mmol) were added. The colorless mixture first became yellow and then brown, and no starting material was detected after 3 h by TLC (E). It was concentrated and the residue chromatographed (E containing 1% of triethylamine) to give 913.4 mg (97%) of pure syrupy **22**: R_f 0.80 (E); $[\alpha]_D^{20}$ +94.5 (c = 0.93 $CHCl_3$). Anal. ($C_{15}H_{15}N_4O_2Cl_3$) C, H, N, Cl.

Preparation of (1*S*,2*S*,3*S*,4*R*,6*R*)-3-Benzyloxy-4,6-diazido-1,2-dihydroxycyclohexane (25) and (1*R*,2*S*,3*S*,4*R*,6*S*)-3-Benzyloxy-1,4-diazido-2,6-dihydroxycyclohexane (26). A stirred mixture of the anhydrocyclitol **24**[14] (190.5 mg, 0.73 mmol) and sodium azide (142.2. mg, 2.18 mmol) in dry *N*,*N*-dimethylformamide (7 mL) was boiled under reflux. After completion of the reaction (3 h) the solvent was distilled off by means of coevaporation with toluene. The residue was dissolved in dichloromethane (15 mL) and the solution washed with water (2 × 5 mL). The organic layer was dried ($MgSO_4$), concentrated, and the residue was submitted to column chromatography (G→B) to give, as the major product, crystalline **25** (145.2 mg, 65.4%) and syrupy **26** (10.6 mg, 4.8%) as the minor component. Compound **25**: mp 82−83 °C; $[\alpha]_D^{20}$ = +24.2 (c = 1.02 $CHCl_3$); R_f 0.6 (H). Anal. ($C_{13}H_{16}N_6O_3$) C, H, N. Compound **26**: $[\alpha]_D^{20}$ = +10.2 (c = 0.8 $CHCl_3$); R_f 0.8 (H). Anal. ($C_{13}H_{16}N_6O_3$) C, H, N.

Enzyme Inhibitory Assay. Preparation of PI4K55 and PI4K230. The enzymes PI4K55 and PI4K230 were isolated and purified from bovine brain membranes as described by Gehrmann et al.[29] The specific activity of the PI4K230 preparation was 3000 U·mg^{-1} and SDS−PAGE showed a major protein band of 200 kDa. The PI4K55 preparation showed a specific activity of about 40 U·mg^{-1} and was free from other isoforms as revealed by using immunoblots.[27]

Assay of PI4K Activity. PI4K activity was routinely tested at 25 °C in a 38-μL volume containing 0.83 mg/mL PtdIns, 5 mM [γ^{32}-P]ATP (400−800 Bq/nmol), 27 mM $MgCl_2$, 116 mM KCl, 116 mM HEPES/KOH, 1 mM EDTA, 1 mM EGTA, 1 mM DTE and 0.4% Triton X-100, pH 7.5, as described by Varsányi et al.[30] One unit of enzyme activity was defined as the amount of the enzyme which catalyzes the formation of 1 nmol PtdIns-4P/min at 25 °C.

Inactivation of PI4Ks with Cyclitol Derivatives. The cyclitol derivatives were dissolved in acetonitrile or dimethyl sulfoxide at a concentration of 100 mM. This resulting solution was further diluted with ethylene glycol to 26 mM. Routinely, 1 μL of the cyclitol derivative was added to 25 μL of the PI4K preparation (10−30 U/mL in 10 mM K-phosphate/150 mM NaCl/0.5 mM dithioerythritol/0.05% Triton X-100, pH 7.0) and incubated for 10 min at 25 °C. Then the reaction mixture was supplemented with the substrate solution and incubated for a further 5−10 min at 25 °C to determine the residual activity. The composition of the inactivation reaction was routinely 1 mM cyclitol derivative, 1% acetonitrile or DMSO, and 3% ethylene glycol. By varying the concentrations of the inhibitory

compounds the composition of the solvent mixture was kept constant. The control experiments carried out without the cyclitol compounds resulted in 10−15% loss of the enzyme activity during the experimental period. The enzyme activities were expressed as a percent (%) of the appropriate controls. The reactions were run in duplicate or triplicate, and the average of two or three independent experiments was calculated. SEM was less than 5% of the means.

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