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# Synthesis of 1L-*chiro*-Inositol 2,3,5-Trisphosphorothioate, the First Partial Agonist at the Platelet *myo*-Inositol 1,4,5-Trisphosphate Receptor

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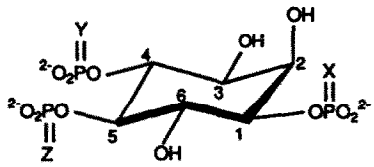
**Abstract**—The synthesis of L-*chiro*-inositol 2,3,5-trisphosphorothioate, a novel analogue of the second messenger D-*myo*-inositol 1,4,5-trisphosphate has been accomplished from the natural product L-quebrachitol. Phosphitylation of (–)-1L-1,4,6-tri-*O*-benzoyl-*chiro*-inositol obtained from L-quebrachitol followed by sulfoxidation of the products gave (–)-1L-1,4,6-tri-*O*-benzoyl-*chiro*-inositol 2,3,5-tris[di(2-cyanoethyl) phosphorothioate], which was deblocked using sodium in liquid ammonia to give 1L-(–)-*chiro*-inositol 2,3,5-trisphosphorothioate. 1L-*chiro*-Inositol 2,3,5-trisphosphorothioate is a partial agonist in the release of intracellular $Ca^{2+}$ from saponin-permeabilised platelets and is both a key tool for pharmacological dissection of the polyphosphoinositide pathway of cellular signalling and a lead compound for the design of small molecule Ins(1,4,5)$P_3$ receptor antagonists.

## Introduction

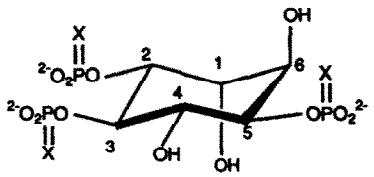
Within the last decade it has become clear that D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$] (**1**, Figure 1) is the second messenger that mobilises $Ca^{2+}$ from endoplasmic reticular stores in stimulated cells. This action is thought to underlie the complex $Ca^{2+}$ signals evoked by many cell-surface receptors linked to phosphoinositidase C, and Ins(1,4,5)$P_3$ on binding to an intracellular receptor initiates $Ca^{2+}$ release through an integral ion channel.[1–3] The cerebellar Ins(1,4,5)$P_3$ receptor [Ins(1,4,5)$P_3$R] has now been cloned and there is evidence for multiple forms arising either from separate genes or from alternative mRNA splicing.[4,5] Consequently, there is speculation on the possibility of a diversity of ligand affinities and/or regulation of the $Ca^{2+}$ channel.[5] Elucidation of the mechanisms regulating $Ca^{2+}$ release by such intracellular receptors is however still hampered by a lack of suitable pharmacological agents, especially small molecules.[6,7] Heparin' and decavanadate are the only Ins(1,4,5)$P_3$ antagonists currently known and are large complex molecules that are generally unsuitable because of their lack of specificity.[4,8]

The search for a specific ligand with low, or preferably no, intrinsic activity is thus of considerable importance. However, virtually all active inositol polyphosphates and analogues examined to date (synthetic and endogenous) appear to be full agonists with respect to Ins(1,4,5)$P_3$ when assessed by the extent that they mobilise $Ca^{2+}$ stores.[4,6] A possible exception to this general rule is inositol 1,3,4,6-tetrakisphosphate [Ins(1,3,4,6)$P_4$], which appears to be a partial agonist[9] of high intrinsic activity.



(1)

X=Y=Z=O (1)
X=Y=Z=S (2)
X=O; Y=Z=S (3)
X=Y=O; Z=S (4)
X=S; Y=Z=O (5)



(2)

X=O (6)
X=S (7)

**Figure 1.** Structures of Ins(1,4,5)$P_3$ and synthetic analogues.

We have focused upon the synthesis of inositol polyphosphate analogues, for example, the metabolically stable phosphorothioates such as *myo*-inositol 1,4,5-trisphosphorothioate [Ins(1,4 5)$PS_3$][6,7,10–12] (**2**, Figure 1), *myo*-inositol 1-phosphate 4,5-bisphosphorothioate (**3**),[13] *myo*-inositol 1,4-bisphosphate 5-phosphorothioate (**4**),[14] and *myo*-inositol 1-phosphorothioate 4,5-bisphosphate (**5**),[15] and ring-modified analogues, such as L-*chiro*-inositol 2,3,5-trisphosphate [L-*ch*-Ins(2,3,5)$P_3$] (**6**)[16,17†] and 2,2-difluoro-2-deoxy inositol 1,4,5-trisphosphate[18] as well as inositol polyphosphates with varying regiochemistry

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†Note the different conventional numbering of the *myo*-and *chiro*-inositol systerns (Figure 1).

of phosphate substitution, such as *myo*-inositol 1,4,6-trisphosphate.[19] All such compounds are fully efficacious analogues in $Ca^{2+}$-mobilising activity, although similar or slightly less potent than Ins(1,4,5)$P_3$.[16,20]

We report here the synthesis of the phosphorothioate derivative of L-*ch* Ins(2,3,5)$P_3$, revealing in L-*chiro*-inositol 2,3,5-trisphosphorothioate [L-*ch*-Ins(2,3,5)$PS_3$][21] (**7**, Figure 1) the first evidence of a ligand possessing very low intrinsic activity at the platelet Ins(1,4,5)$P_3$ receptor.

## Results and Discussion

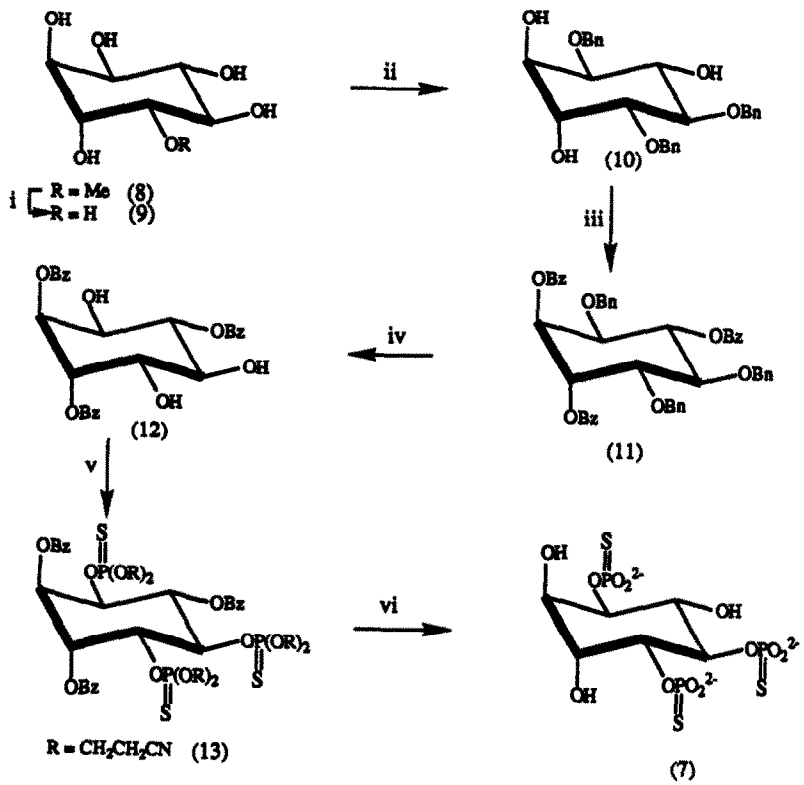
With the development of inositol polyphosphate synthesis within the last five years the synthesis of analogues of Ins(1,4,5)$P_3$ designed to probe the relationship between ligand structure, receptor occupation and response has become possible.[7,10,11] While useful in determining the structural requirements for active ligands, our understanding of the occupation–response relationship at Ins(1,4,5)$P_3$R(s) is still limited and it is unclear for example what apparent binding affinity values represent in radioligand assays, since they are generally lower than $EC_{50}$ values for $Ca^{2+}$ release for most ligands.[4] It is also not clear whether the binding affinities could represent interaction with conformations other than the active Ins(1,4,5)$P_3$R. The binding of labelled Ins(1,4,5)$P_3$ to membranes reveals a high affinity, slowly dissociating site that is totally inconsistent with the kinetics estimated from $Ca^{2+}$ flux experiments.[4] Moreover, recent experiments show that under conditions of low [$Ca^{2+}$] a lower affinity rapidly dissociating component of Ins(1,4,5)$P_3$ binding may represent labelling of the active form of the Ins(1,4,5)$P_3$R in hepatocyte membranes.[24]

The complexity of $Ca^{2+}$ release through the Ins(1,4,5)$P_3$ receptor channel appears to result from both extra- and intra-luminal regulatory features.[4] The ability of a $Ca^{2+}$ store to respond rapidly, but transiently, to low concentrations of Ins(1,4,5)$P_3$, while still maintaining its ability to release in response to higher concentrations has been termed 'quantal'[25,26] and complicates the relationship between Ins(1,4,5)$P_3$ receptor occupation and release and the ability to distinguish agonists of different efficacy. These complications have probably resulted in virtually all Ins(1,4,5)$P_3$ analogues to date displaying apparent full agonist properties, since even partial agonists may be able to deplete stores fully, given sufficient time. The naturally occurring Ins(1,4,5)$P_3$ metabolite, *myo*-inositol 1,3,4,6-tetrakisphosphate, Ins(1,3,4,6)$P_4$, releases intracellular stores of $Ca^{2+}$ from permeabilised cells with indications of partial agonist activity in comparison to Ins(1,4,5)$P_3$,[27] releasing > 80% of the $Ca^{2+}$ mobilised by Ins(1,4,5)$P_3$ in SH-SY5Y cells.[27] We found no evidence of partial agonism for Ins(1,3,4,6)$P_4$ in platelets (data not shown). The synthetic Ins(1,4,5)$P_3$ analogue, L-*ch*-Ins(2,3,5)$P_3$[17] is a full agonist at the Ins(1,4,5)$P_3$ receptor,[16] but is less potent than Ins(1,4,5)$P_3$. We demonstrate here clear partial agonist activity displayed by the synthetic phosphorothioate L-*ch*-Ins(2,3,5)$PS_3$ as determined by the extent of $Ca^{2+}$ released from permeabilised platelets. This probably indicates that this agent possesses very low intrinsic efficacy at the Ins(1,4,5)$P_3$R, only providing a minute $Ca^{2+}$ leak relative to the $Ca^{2+}$ pump and has been discussed.[21]

We synthesised L-*ch*-Ins(2,3,5)$PS_3$ by a modification of our approach to L-*ch*-Ins(2,3,5)$P_3$.[17] Thus, L-quebrachitol (**8**) was demethylated to 1L-*chiro*-inositol (**9**) (Scheme I) and treatment of the latter with dibutyltin oxide and tetrabutylammonium iodide in acetonitrile formed a dibutylstannylene derivative which on benzylation gave 1L-2,3,5-tri-*O*-benzyl-*chiro*-inositol (**10**) as the major product. Treatment of (**10**) with an excess of benzoyl chloride in pyridine gave the corresponding 1,4,6-tribenzoate (**11**) as a syrup in quantitative yield. Catalytic hydrogenolysis (Pd/C) of (**11**) gave crystalline 1,4,6-tribenzoate (**12**) (94 %).[17] Phosphitylation of (**12**) using bis(2-cyanoethyl)diisopropylaminophosphine gave the corresponding trisphosphite and sulfoxidation using sulfur in pyridine afforded the fully protected trisphosphorothioate (**13**). Deblocking using sodium in liquid ammonia[12] gave 1L-*chiro*-2,3,5-trisphosphorothioate (**7**), which was purified by ion-exchange chromatography on DEAE Sephadex A-25 resin.

The activation of platelets by agents such as thrombin, platelet activating factor and thromboxane $A_2$ leads to the formation of inositol phosphates and the elevation of cytosolic $Ca^{2+}$ levels.[23] The role of Ins(1,4,5)$P_3$ in $Ca^{2+}$ mobilisation in platelets is well established and $InsP_3$-induced $Ca^{2+}$ release in platelet membrane preparations has been widely demonstrated.[28,29] In addition, evidence for $InsP_3$-induced platelet functional responses is available where Ins(1,4,5)$P_3$ can induce platelet aggregation[30] and dense granule release.[31] The localisation of the Ins(1,4,5)$P_3$-binding site has been shown to be on the intracellular membranes of platelets[32] and indicates the presence of an intracellular receptor which is consistent with studies on other tissues. To date, studies concerning $Ca^{2+}$ release have concentrated on the use of purified membrane preparations and membrane vesicles. We report here the first $Ca^{2+}$ release assay involving the use of permeabilised platelets.

L-*ch*-Ins(2,3,5)$PS_3$ was evaluated as a $Ca^{2+}$ mobilising agonist in permeabilised platelets, relative to Ins(1,4,5)$P_3$. Ins(1,4,5)$P_3$ induced a dose-dependent release of $^{45}Ca^{2+}$ with $EC_{50}$ 1.14 ± 0.19 μM (Figure 2). L-*ch*-Ins(2,3,5)$P_3$ was a full agonist with an $EC_{50}$ of 1.9 ± 0.1 μM. These results contrast with a previous study[16] in SH SY5Y neuroblastoma cells which show L-*ch*-Ins(2,3,5)$P_3$ to be a full agonist, but some 12-fold less potent than Ins(1,4,5)$P_3$. The results shown in Figure 2 indicate clearly that L-*ch*-Ins(2,3,5)$PS_3$ behaves as a partial agonist. These data are very encouraging in our attempts to develop high-affinity Ins(1,4,5)$P_3$ ligands with negligible efficacy. The introduction of phosphorothioate groups appears to result in a substantial fall in agonist efficacy. Ins(1,4,5)$PS_3$ has only a slightly lower affinity than Ins(1,4,5)$P_3$ at [$^3$H] Ins(1,4,5)$P_3$ binding sites.[33,34]




**Scheme I.** Synthesis of L-*chiro*-Ins(2,3,5)$PS_3$.

**Reagents and conditions:** i, 47 % aq HI; ii, (a) $Bu_2$-SnO, $Bu_4NI$-MeCN, (b) BnCl, reflux; iii, BzCl-pyridine; iv, $H_2$-$PdCl_2$/C (5 %) in EtOH; v, (a) $Pr^i_2NP(OCH_2CH_2CN)_2$, tetrazole in $CH_2CH_2$ (b) Sulphur in pyridine; vi, (a) Na/liq $NH_3$, (b) $H_2O$.

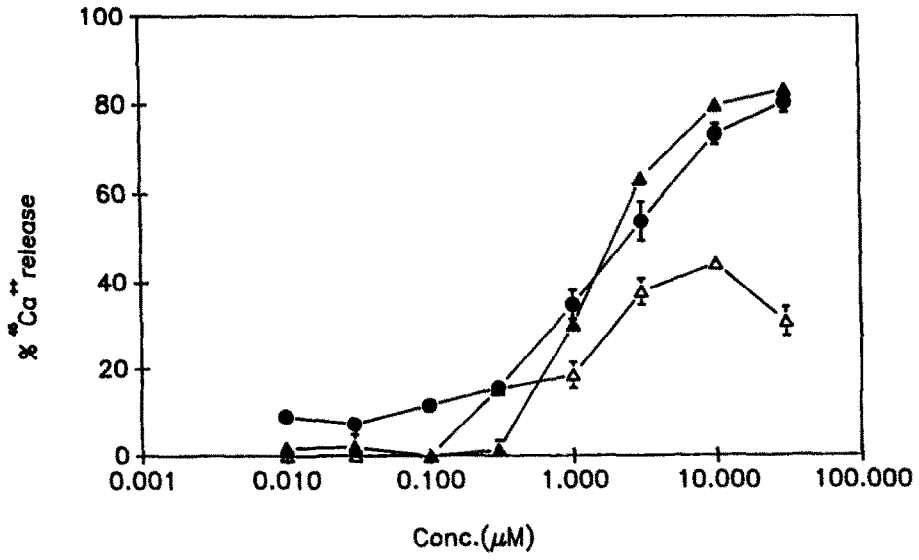



**Figure 2.** The dose–response curves of Ins(1,4,5)$P_3$, L-*chiro*-Ins(2,3,5)$P_3$ and L-*chiro*-Ins(2,3,5)$PS_3$ for their ability to release $^{45}Ca^{2+}$ from permeabilised rabbit platelets at 20 °C. Each point is mean ± δ SE; each point is the mean ± SE of 3 determinations. Ins(1,4,5)$P_3$ (●), L-*chiro*-Ins(2,3,5)$PS_3$ (▲), L-*chiro* -Ins(2,3,5)$PS_3$ (Δ).

Unlike heparin and decavanadate, it is likely that inositol-based Ins(1,4,5)$P_3$ receptor antagonists will be much more specific. Of all the phosphorothioate analogues of inositol polyphosphates synthesised to date this is the first to show other than full agonist activity at useful potency. D-6-Deoxy-Ins(1,4,5)$PS_3$ is a low potency partial agonist in SHSY5Y cells.[21] While the reasons behind this are not yet clear, it is obvious that phosphorothioate substitution leads to changes in molecular size, hydrophobicity and charge distribution, any or all of which may contribute to

producing a less than maximal response. It should be noted that for the second messenger 3',5'-cyclic AMP, phosphorothioate substitution has generated the only known competitive cyclic AMP antagonists in $R_p$-cyclic AMPS[35] and cyclic $AMPS_2$[36] out of many hundreds of synthetic cyclic AMP analogues.

The development of a low intrinsic activity partial agonist is clearly a significant development in delineating structure–activity relationships at Ins(1,4,5)$P_3$R(s) and could prove useful in revealing the mechanisms of complex $Ca^{2+}$ signalling in cells and in the development of more effective pharmacological tools to probe the polyphosphoinositide pathway of signal transduction.

## Experimental

Ins(1,4,5)$P_3$ was obtained from the University of Rhode Island Foundation Chemistry group, as its $K^+$ salt. Ins(1,3,4,6)$P_4$ was obtained from Calbiochem UK. L-*chiro*-Ins(2,3,5)$P_3$ was synthesised as previously described[17] and used as its triethylammonium salt. TLC was performed on silica gel 60F: (Merck) with detection by UV light or with methanolic phosphomolybdic acid. Flash-column chromatography was performed on silica gel (SORBSIL C60). The $^1$H-NMR spectra (internal $Me_4Si$) were recorded with Bruker AM-300 or Jeol JMN-GX270 or JMN-GX400 spectrometers. The $^{31}$P-NMR spectra (reference, external aqueous 85 % phosphoric acid) were recorded with Jeol FX-90Q or JMN-GX400 or Bruker AM-300 spectrometers. Mass spectra were recorded at the SERC Mass Spectrometry Service Centre (Swansea) or at the Mass Spectrometry Service, University of Bath. Microanalysis was carried out at Butterworth Laboratories Ltd or by the Microanalysis Service, University of Bath. Melting points (uncorrected) were determined using a Reichert–Jung Thermo Galen Kofler Block. Optical rotations at 589 nm were measured with an Optical Activity Ltd Polarimeter Type-AA-10. Ion-exchange chromatography was performed on DEAE Sephadex A-25 eluting with a gradient of triethylammonium hydrogen carbonate (TEAB) buffer at pH 8.0. Quantitative analysis of phosphate was performed using the Briggs phosphate assay.[22]

### *$^{45}Ca^{2+}$ Release*

Rabbit platelets were isolated and washed according to Murphy *et al.*[23] The platelets ($10^9$/mL) were resuspended in an 'intracellular-like' buffer, permeabilised with 40 μg/mL saponin for 1 min, washed and loaded with $^{45}Ca^{2+}$ for 60 min. 100 μL Aliquots of permeabilised cells were then challenged with Ins(1,4,5)$P_3$ or analogue for 3 min at 20 °C and the cell associated $^{45}Ca^{2+}$ determined by rapid filtration of the platelet suspension/agonist mixture through GF-B filter strips on a Brandell cell harvester. Aliquots of platelets were also treated with vehicle (0 % $^{45}Ca^{2+}$ release) or 30 μM inomycin (100 % release) and all results were expressed as a percentage of ionomycin releasable $^{45}Ca^{2+}$.

### *(–)-1,4, 6-Tri-*O*-benzoyl-1L-*chiro*-inositol-2,3,5-tris[di-(2-cyanoethyl)phosphorothioate] (13)*

To a mixture of **12** (0.098 g, 0.2 mmol) and 1H-tetrazole (0.125 g, 1.8 mmol) in dry dichloromethane (5 ml), bis(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (0.41 g, 1.8 mmol) was added. The mixture was stirred at room temperature for 1 h and concentrated. The resulting residue was dissolved in dry pyridine (5 ml) and sulphur (0.1 g) was added. The mixture was stirred at room temperature for 5 h, the pyridine was evaporated, the residue was dissolved in $CHCl_3$ and the solution was washed with saturated aqueous $NaHCO_3$ (10 ml), dried ($MgSO_4$), and concentrated. Flash-column chromatography of the residue [light petroleum (40–60 °)–EtOAc] gave **8** (0.19 g, 90 %), isolated as an oil, $[\alpha]_D^{20}$ –13 ° (*c* 3.0, chloroform). NMR data ($CDCl_3$): $^1$H (270 MHz), δ 2.62–2.77 (m, 12H, $CH_2$), 4.16–4.26 (m, 12H, $CH_2$), 5.30–5.38 (m, 3H, 3CH), 5.93–6.99 (m, 3H, 3 CH), 7.27–7.69 (m, 9H, Ph), 8.11–8.25 (m, 6H, Ph); $^{31}$P (162 MHz), δ 66.90 and 68.04. Mass spectrum: m/z (FAB) 1099 ($M^+$, 2 %), 879 (4), 608 (11), 391 (3), 377 (3), 224 (20), 149 (12), 105 (100). Accurate Mass for $C_{45}H_{45}N_6O_{15}P_3S_3$ 1098.132; Found 1098.132.

### *1L-(–)-*chiro*-Inositol 2,3,S-trisphosphorothioate (7)*

To liquid ammonia (40 ml), a solution of **13** (0.055 g, 0.05 mmol) in dry dioxane (1.8ml) was added, followed by sodium (0.1 g, 4.3 mmol) in small pieces. The solution was stirred for 5 min, the reaction was quenched with ethanol, and the ammonia was evaporated in a stream of nitrogen, and concentrated. Ion-exchange chromatography of the residue on DEAE Sephadex A-25 using a gradient from $H_2O$ to M TEAB (pH 8.0) gave **7** (0.035 mmol, 70 %). **7** Was eluted at *ca* 800 μM TEAB, $[\alpha]_D^{20}$ –10 ° (*c* 2.5, methanol). NMR data ($D_2O$): $^1$H (270 MHz), δ 3.85 (dd, 1H, *J* 9.8 Hz, CH), 3.94 (dd, 1H, *J* 9.9 Hz, CH); 4.25–4.55 (m, 4H, 4CH); $^{31}$P (121.5 MHz), δ 42.20, 43.81 and 46.97. Mass spectrum: (FAB) m/z 466 [$M^-$, 100 %], 432 (20), 399 (8), 353 (9), 337 (10), 188 (15), 113 (13), 95 (25). Accurate Mass for $C_6H_{14}O_{12}P_3S_3$ 466.886; Found 466.887.

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## References

1. Berridge, M. J. *Nature* (Lond.) **1993**, *361*, 159.

2. Berridge, M. J. *J. Biol. Chem.* **1990**, *265*, 9583.

3. Jacob, R. *Biochem. Biophys. Acta* **1990**, *1052*, 427.

4. Taylor, C. W.; Richardson, A. *Pharmacol. Ther.* **1991**, *51*, 97.

5. Ferris, C. D.; Snyder, S. H. *J. Neurosci.* **1992**, *12*, 1567.

6. Nahorski, S. R.; Potter, B. V. L. *Trends Pharmacol. Sci.* **1989**, *10*, 139.

7. Potter, B. V. L. *Nat. Prod. Rep.* **1990**, *7*, 1.

8. Strupish, J.; Wojcikiewicz, R. J. H.; Challis, R. A. J.; Safrany, S. T.; Willcocks, A. L.; Potter, B. V. L.; Nahorski, S. R. *Mol. Neuropharmacol.* **1991**, *1*, 111.

9. Gawler, D. J.; Potter, B. V. L.; Grigg, R.; Nahorski, S. R. *Biochem. J.* **1991**, *276*, 163.

10. Potter, B. V. L.; Nahorski, S. R. *Biochem. Soc. Trans.* **1992**, *20*, 434.

11. Potter, B. V. L. In *Drug Design for Neuroscience*, pp 383–416, Kozikowski, A. P., Ed.; Raven Press; New York 1993.

12. Cooke, A. M.; Gigg, R.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1987**, 1525.

13. Noble, N. J.; Dubreuil, D.; Potter, B. V. L. *BioMed. Chem. Lett.* **1992**, *2*, 471.

14. Noble, N. J.; Cooke, A. M.; Potter, B. V. L. *Carbohydr. Res.* **1992**, *234*, 177.

15. Lampe, D.; Mills, S. J.; Potter, B. V. L. *J. Chem. Soc. Perkin Trans. I* **1992**, 2899.

16. Safrany, S. T.; Wilcox, R. A.; Liu, C.; Potter, B. V. L.; Nahorski, S. R. *Eur. J. Pharmacol. (Mol. Pharmacol. Section)* **1992**, *226*, 265.

17. Liu, C.; Nahorski, S. R.; Potter, B. V. L. *Carbohydrate Res.* **1992**, *234*, 107.

18. Sawyer, D. A.; Potter, B. V. L. *J. Chem. Soc. Perkin Trans. I* **1992**, 923.

19. Mills, S. J.; Al-Hafidh, J.; Westwick, J.; Potter, B. V. L. *BioMed. Chem. Lett.* **1993**, *3*, 2599.

20. Strupish, J.; Cooke, A. M.; Potter, B. V. L.; Gigg, R.; Nahorski, S. R. *Biochem. J.* **1988**, *253*, 901.

21. Safrany, S. T.; Wilcox, R. A.; Liu, C.; Dubreuil, D.; Potter, B. V. L.; Nahorski, S. R. *Mol. Pharmacol.* **1993**, *43*, 499.

22. Briggs, A. *J. Biol. Chem.* **1922**, *53*, 13.

23. Murphy, C. T.; Elmore, M. ; Kellie, S.; Westwick, J. *Biochem. J.* **1991**, *278*, 255.

24. Rouxel, F. P.; Hilly, M.; Mauger, J.-P. *J. Biol. Chem.* **1992**, *267*, 20017.

25. Muallem, S.; Pandol, S. J.; Beeker, T. G. *J. Biol. Chem.* **1989**, *264*, 205.

26. Taylor, C. W.; Potter, B. V. L. *Biochem. J.* **1990**, *266*, 189.

27. Gawler, D. J.; Potter, B.V. L.; Nahorski, S. R. *Biochem. J.* **1991**, *276*,163.

28. Rengasamy, A.; Feinberg, H. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 1021.

29. Authi, K. S.; Crawford, N. *Biochem. J.* **1988**, *230*, 247.

30. Knezevic, I.; Dieter, J. P.; Le Breton, J. C. *J. Pharm. Exp. Ther.* **1992**, *260*, 947.

31. Brass, L. F.; Shaller, C. C.; Belmonte, E. G. *J. Clin. Invest.* **1987**, *79*, 1269.

32. Authi, K. S. *FEBS Lett.* **1992**, *298*, 173.

33. Nunn, D.; Taylor, C. W. *Biochem. J.* **1990**, *270*, 227.

34. Willcocks, A. L.; Potter, B. V. L.; Cooke, A. M.; Nahorski, S. R. *Eur. J. Pharmacol.* **1988**, *155*, 181.

35. Parker Botelho, L. H.; Rothermel, J. D.; Coombs, R. V.; Jastorff, B. *Meth. Enzymol.* **1988**, *159*, 159.

36. Parker Botelho, L. H.; Baraniak, J. W.; Stec, W. J. *J. Biol. Chem.* **1988**, *263*, 5301.

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