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Synthesis and evaluation of a phosphonate analogue of the soluble guanylate cyclase activator YC-1

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Abstract—Soluble guanylate cyclase (sGC) is activated by the known benzylindazole derivative YC-1 [1-benzyl-3-(5′-hydroxymethyl-2′-furyl)-indazole]. YC-1 also acts synergistically with CO, activating sGC to a level comparable to that achieved upon binding of nitric oxide, the endogenous activator of sGC. We here describe the synthesis of a YC-1 phosphonate analogue with improved aqueous solubility as well as its effects on sGC.

Soluble guanylate cyclase (sGC)[1] is a heterodimeric hemoprotein consisting of an α1 and β1 subunit. sGC is the primary nitric oxide (NO) receptor in mammals and is activated several hundred-fold over the basal activity upon the binding of NO, catalyzing the conversion of GTP to cGMP and pyrophosphate. cGMP is required for vascular smooth muscle relaxation, neuronal signaling, and platelet aggregation.[1–4] Compounds capable of activating sGC present an approach to treating diseases involving the NO-cGMP pathway.

In addition to NO activation, sGC can also be activated 3- to 5-fold upon binding of carbon monoxide (CO) to the heme, or 8- to 12-fold in the presence of the benzylindazole derivative YC-1 [3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole] (Fig. 1a), a known activator of sGC.[5] Furthermore, YC-1 and CO together act synergistically to activate the enzyme to a level similar to that observed with NO.[6,7] A variety of experimental approaches, aimed at locating the YC-1 binding site within sGC, have been inconclusive.[8–14] YC-1 binding in the heme pocket on the β1 subunit,[9,10] to a predicted PAS domain on the α1 subunit,[12] and to a regulatory GTP binding site on the catalytic domains (α1 and β1 subunits)[15,16] have all been proposed. The conflicting results of these studies leave YC-1's precise mode of action an open question.

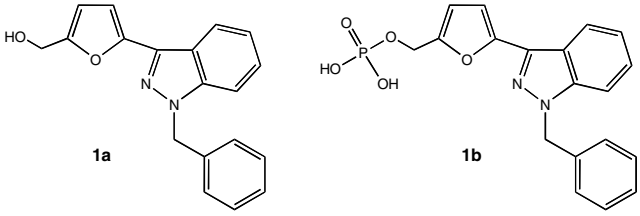

Figure 1. (a) YC-1 and (b) YC-1 phosphate analogue initially pursued.

To further explore the effects of YC-1 on sGC, a phosphate analogue (Fig. 1b) was initially pursued. The rationale behind the phosphate analogue was twofold: (1) while YC-1 has been shown to be efficacious in vivo, its aqueous solubility is extremely low (<200 μM). Phosphorylation of the YC-1 hydroxy group would be expected to improve solubility. (2) Also, the analogue could be used to test the theory that YC-1 is a nucleotide mimic capable of activating sGC by binding to a 'pseudosymmetric' site.[15,17] If YC-1 binds to a substrate binding site, phosphorylation of YC-1 may provide a better mimic thus increasing the potency of the activator. Phosphorylation of the YC-1 hydroxy group was therefore attempted to address both of these factors.

Initial attempts at the phosphorylation of YC-1 employed standard approaches. Treatment with phospho-

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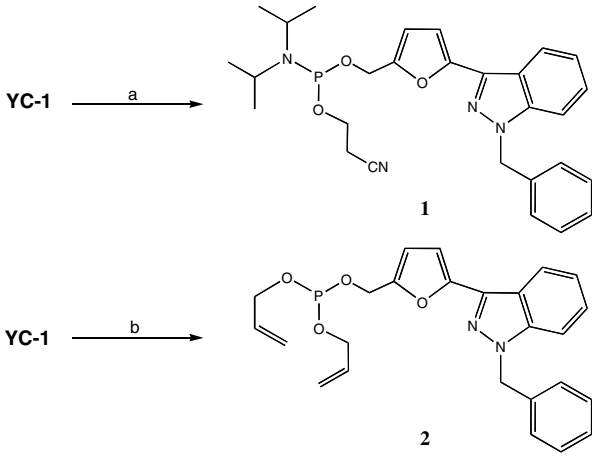
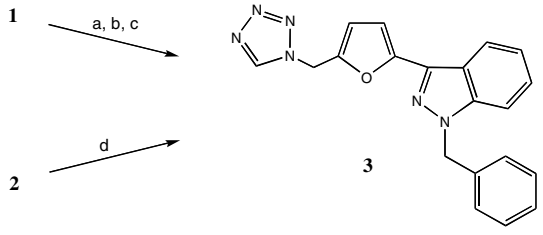


Scheme 1. Conversion of YC-1 to phosphoramidite intermediate **1** and phospho-triester intermediate **2**. Reagents: (a) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, MeCN; (b) diallyl *N*,*N*-diisopropylphosphoramidite, tetrazole, CH_2Cl_2.

rus oxychloride followed by aqueous work-up failed to yield the desired product. Other attempts involving the low temperature deprotonation followed by treatment with tetrabenzyl pyrophosphate were also unsuccessful. As a third option phosphoramidite chemistry was then explored. While not successful in yielding the desired phosphorylated YC-1 species, the phosphoramidite approach revealed a unique pattern of reactivity within the molecule. Treatment of YC-1 with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite or diallyl *N*,*N*-diisopropylphosphoramidite successfully yielded the expected phosphoramidite **1** or phosphite-triester **2** intermediates (Scheme 1).

In the attempted oxidative conversion of both **1** and **2** to their respective phosphate derivatives however, the unexpected product **3** was obtained (Scheme 2).

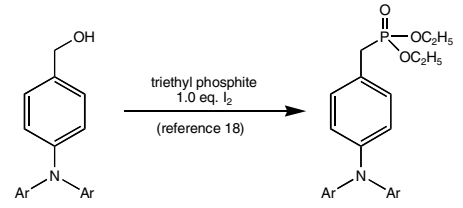


Scheme 2. Conversion of compounds **1** and **2** to unexpected adduct **3**. Reagents: (a) tetrazole, $MeCN/H_2O$; (b) I_2, $THF/Pyr/H_2O$; (c) DBU; (d) mCPBA.

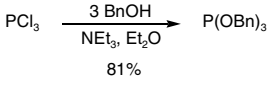
While compounds **1** and **2** are stable and observable by NMR it seems that upon oxidation to the phosphate a rapid elimination/addition process occurs. The elimination of the phosphate is likely aided by the nitrogen lone-pair present in the extended conjugated system. This elimination product is then quenched by an available nucleophile, in this case residual tetrazole (Scheme 3).

Based upon these findings it seems unlikely that phosphorylation of the YC-1 hydroxy group is possible. The insights gained into the inherent reactivity of the molecule itself however suggested that a phosphonate analogue might be accessible by a modified Michaelis–Arbuzov approach. It was previously shown by the group of Marder[18] that very electron-rich, benzylic-type, phosphonates can be prepared by treatment of the corresponding alcohol in triethyl phosphite with one equivalent of iodine in a general one-pot process (Scheme 4).

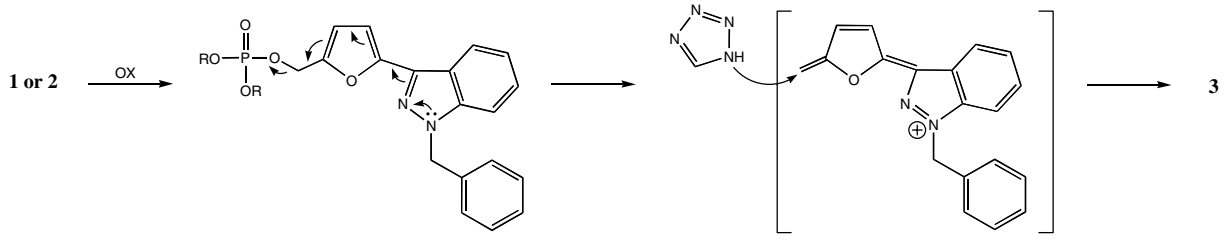
This approach to a YC-1 phosphonate analogue seemed appealing with the exception of the use of triethyl phosphite. Diethyl phosphonates typically require harsh (Lewis or protic acid) hydrolysis conditions to yield the deprotected phosphonate.[19] To circumvent this issue we chose to investigate the use of tribenzyl phosphite in the iodine-promoted preparation of a dibenzyl phosphonate analogue of YC-1. The resulting dibenzyl phosphonate could then be deprotected using milder hydrogenation conditions. Tribenzyl phosphite[20] is not commercially available and was readily prepared by the treatment of phosphorus trichloride with benzyl alcohol (Scheme 5).



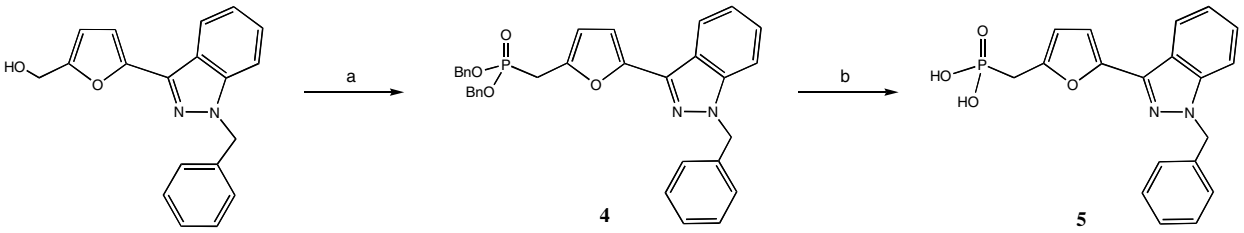
Scheme 4. One-pot preparation of electron-rich phosphonates from the corresponding alcohol.



Scheme 5. Preparation of tribenzyl phosphite.



Scheme 3. Mechanistic rationale for conversion of compounds **1** and **2** to tetrazole adduct **3**.



Scheme 6. Preparation of the phosphonate analogue of YC-1 **5** via dibenzyl phosphonate **4**. Reagents: (a) $P(OBn)_3$, 1.0 equiv I_2; (b) H_2, Pd/C, MeOH.

When treated with an excess of tribenzyl phosphite and one equivalent of iodine at room temperature, YC-1 is slowly converted to the desired dibenzyl phosphonate **4** (conversion accelerated upon heating to 40 °C). As expected, hydrogenation of **4** successfully provided the deprotected YC-1 phosphonate analogue **5** (Scheme 6).

Effect of YC-1 and phosphonate analogue **5** *on sGC activity*. In agreement with our prediction, the YC-1 phosphonate analogue **5** shows improved aqueous solubility (>800 μM) compared with YC-1 (aqueous solubility <200 μM). The ability of the phosphonate analogue **5** to activate sGC was next investigated. Activity assays of YC-1 (at 150 μM) and analogue **5** (at 150 and 800 μM) with sGC gave the results shown in Table 1. At 150 μM, YC-1 and compound **5** both activate sGC (6.4- and 5.3-fold, respectively). In the presence of CO, the same concentrations of YC-1 and compound **5** act synergistically, activating 87-fold and 16-fold, respectively. However, NO induced activity decreased from 77- to 38-fold in the presence of compound **5**. This inhibition is unlike the synergism that is observed with YC-1 and NO.

Somewhat surprisingly, the phosphonate analogue **5** shows much different effects with sGC at the higher concentration tested (800 μM). In all cases this concentration of compound **5** suppressed sGC activity relative to the fold activation observed at a concentration of 150 μM (Table 1). A possible explanation for this effect may be that compound **5** is also able to bind at the catalytic substrate (GTP) binding site, acting as a competitive inhibitor at the higher concentration tested. Also of interest are the differing effects observed for compound **5** with the Fe–NO and Fe–CO complexes of sGC. A possible explanation for this could be that compound **5** has a lower K_d of binding for the GTP binding site in the Fe–NO form of the enzyme. It is well established that the K_m for sGC with its endogenous substrate (GTP) is dependent on the ligation state of the enzyme.[9]

Table 1. Activation of sGC heme complexes by YC-1 and analogue **5**[a]

Compound	Fold activation		
	Fe(II)	Fe(II)–NO	Fe(II)–CO
—	1.0 ± 0.1	77 ± 8.9	2.0 ± 0.1
150 μM YC-1	6.4 ± 2.3	270 ± 36	87 ± 7.5
150 μM **5**	5.2 ± 0.8	38 ± 16	16 ± 2.1
800 μM **5**	1.4 ± 0.1	10 ± 0.6	1.2 ± 0.5

[a] Purified sGC was obtained from a baculovirus/Sf9 expression system as described. Two min end-point assays were performed in duplicate at 37 °C.[21] The final concentration of DMSO was 2% v/v. Basal activity was 90 ± 4 nmol/min/mg ($n = 2$). All experiments with YC-1 and analogue **5** were repeated 3 times to ensure reproducibility.

In conclusion, while the preparation of a phosphorylated YC-1 species was not possible, the information gained from our attempts has shed light onto an inherent reactivity pattern for this important sGC-activating molecule. From the insights gained, a phosphonate analogue of YC-1 was successfully prepared via a modified Michaelis–Arbuzov approach. This analogue shows improved aqueous solubility and a comparable ability to activate sGC at concentrations typically used in the YC-1 activation of sGC. With improved solubility properties the phosphonate analogue was also tested at higher concentrations showing a somewhat unexpected inhibitory effect. Future work will be aimed at establishing the nature of this apparent inhibition.

Supplementary data

Supplementary data associated with this article can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.039.

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