

A CONVENIENT ROUTE TO THE SOLUBLE GUANYLATE CYCLASE ACTIVATOR YC-1 AND ITS N2 REGIOISOMER

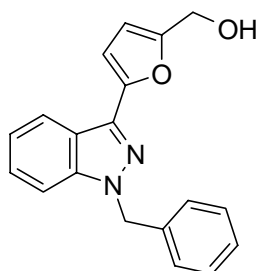
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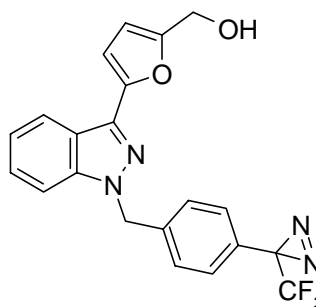
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Abstract – A new route to the soluble guanylate cyclase (sGC) activator YC-1 and its *N*2 regioisomer has been established with a Mitsunobu mediated *N*-alkylation as the key step. The route is utilised in the synthesis of a potential photoaffinity label.

sGC is the only physiological receptor for nitric oxide (NO) discovered hitherto. NO is a pleiotropic signalling molecule with widespread effects in both the cardiovascular and central nervous system.¹ NO activates sGC by binding to a haem cofactor which leads to a conformational change in the enzyme and catalysis of the conversion of guanosine-5'-triphosphate to cyclic guanosine-3',5'-monophosphate.^{2,3}



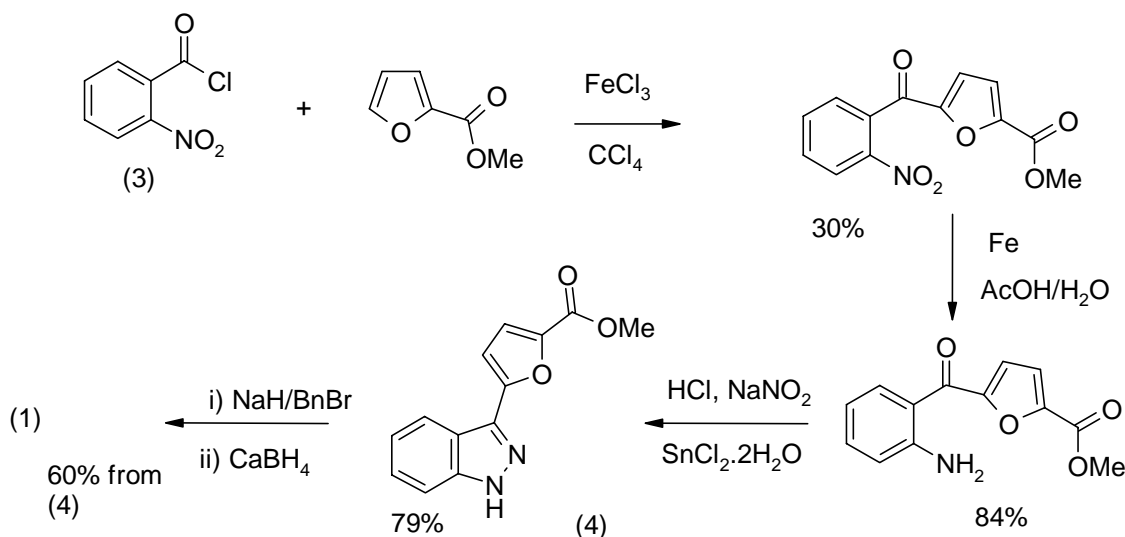
(1)



(2)

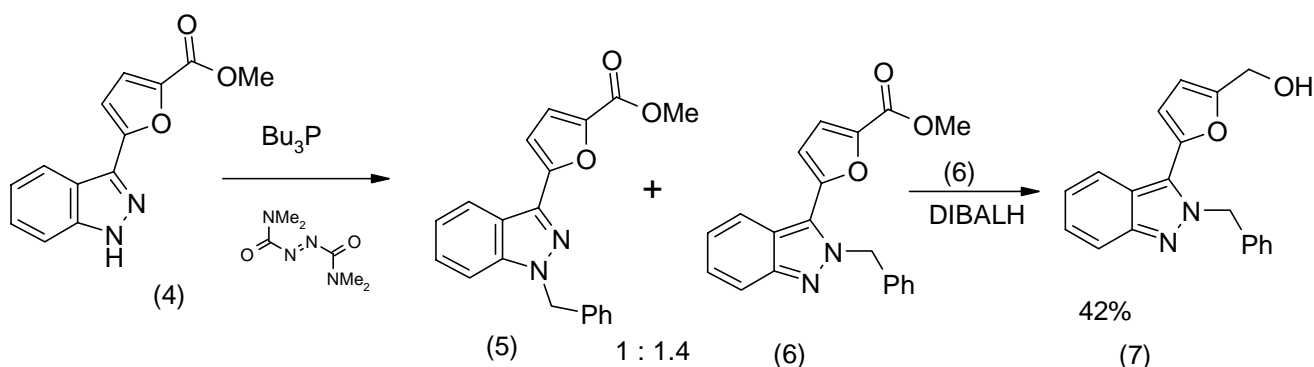
YC-1 (**1**) has been widely reported as an NO-independent activator of sGC,^{4,5,6} though it is also known to be a non-specific inhibitor of phosphodiesterases⁷ and to modulate the activity of Ca²⁺-activated and voltage-dependent K⁺ channels.⁸ In connection with our studies⁹ on novel activators of the sGC enzyme, we required gram quantities of this important pharmacological tool and a convenient route to analogues such as the diazirine (**2**). The original reported synthesis,¹⁰ was in our hands, low yielding and the other

reported methods involved the palladium coupling of organo-tin reagents¹¹ or Suzuki type coupling of 1-benzyl-3-iodoindazole with a furylboronic acid.¹² We chose to construct the indazole heterocycle using classical Friedel-Crafts chemistry to generate an *o*-nitroaryl ketone (**3**), followed by reduction, diazotisation and cyclisation as shown in Scheme 1.



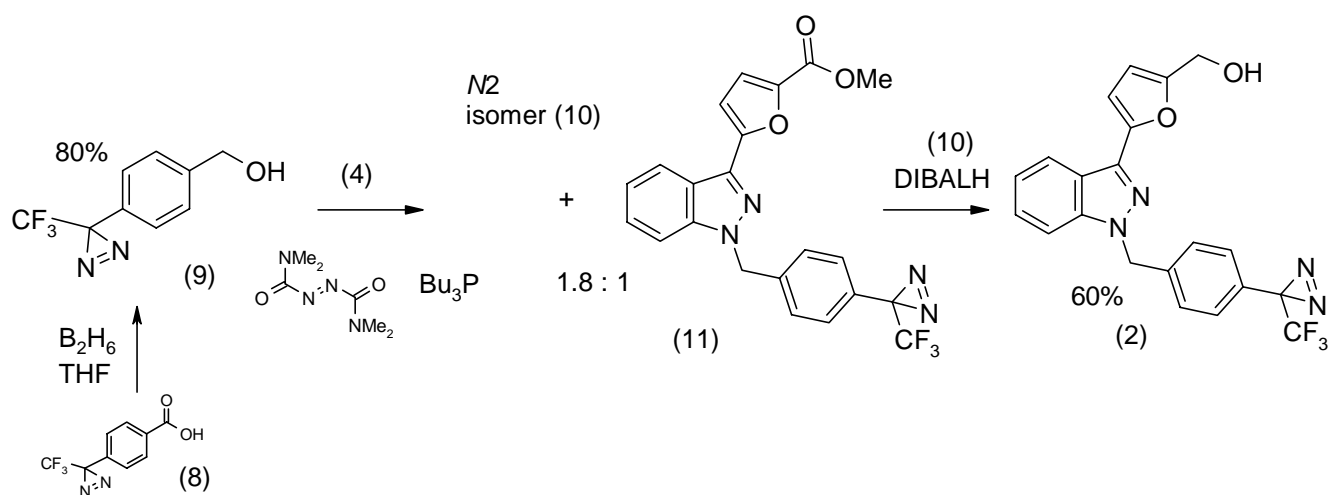
Scheme 1

The synthesis was straightforward, though the yield of the Friedel-Crafts step was only 30%. With gram quantities of the indazole (**4**) now in hand, the benzyl group could be introduced by alkylation and reduction of the ester with CaBH_4 gave YC-1 (**1**). At this point we also desired access to potential photoaffinity labels such as the diazirine (**2**). In this case the preparation of the required substituted benzyl bromide reported in the literature is lengthy.¹³ We therefore turned our attention to the commercially available 4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoic acid. In principle reduction to the alcohol and alkylation of (**4**) using Mitsunobu conditions⁹ would provide the required intermediate. To test the feasibility of the process we conducted the alkylation reaction under modified Tsunoda-Mitsunobu conditions¹⁴ and obtained a mixture of *N*1 and *N*2 regioisomers¹⁵ (**5**) and (**6**) in a ratio of 1:1.4 as shown in Scheme 2.



Scheme 2

This allowed us to prepare the previously unknown *N2* regioisomer of YC-1 (**7**) by DIBALH reduction of **6**. The identity of the regioisomers was established by COSY and NOSEY NMR studies.¹⁶ With this methodology in hand we reduced the diazirine acid (**8**) with diborane in THF and obtained the required alcohol (**9**) (Scheme 3). Mitsunobu reaction gave a mixture of regioisomers (**10**) and (**11**) and reduction now provided the desired diazirine analogue of YC-1. In this case, attempted reduction with CaBH₄ gave only decomposition and the reaction had to be conducted with DIBALH. While YC-1 produced by this route showed similar activation of sGC to that reported previously, neither the *N2* isomer (**7**) nor the diazirine analogue (**2**) showed any significant activation of the enzyme.¹⁷ In summary, we have developed a short synthesis of YC-1 and its analogues and enabled access to the *N2* regioisomers.



Scheme 3

ACKNOWLEDGEMENTS

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- 15 All new compounds gave satisfactory analytical and spectral data. To a solution of **4** (0.1 g, 0.413 mmol) in dry toluene (4 mL) was added Bu₃P (0.2 mL, 0.826 mmol), followed by benzyl alcohol (0.085 mL, 0.826 mmol) and 1,1'-azobis(*N,N*-dimethyl formamide) (0.142 g, 0.826 mmol) and heated at 80°C. After 2 h the reaction mixture was allowed to cool. The precipitated solid was removed by filtration, washed with toluene and the filtrate concentrated *in vacuo*. Column chromatography on silica eluting with cyclohexane/ethylacetate (80:20) gave a mixture of **5** and **6** (0.07 g) as a yellow oil. This mixture was purified by HPLC 40-80% aq. acetonitrile in 0.1% TFA, (HYPERASIL, C18) to give (**6**) as a white solid (18.3 mg, 14 %), mp 112-120°C. δ_H (CDCl₃) 3.9 (3H, s, CO₂CH₃), 5.9 (2H, s, CH₂Ph), 6.75 (1H, d, *J* 3.5, furyl), 7.3 (1H, d, *J* 3.5, furyl), 7.3 (1H, m, *J*₂₋₁ 8.3, *J*₂₋₃ 6.6, *J*₂₋₄ 1.1, indazole), 7.8 (1H, dd, *J*₄₋₃ 8.45, *J*₄₋₂ 1.1, indazole), 7.4 (1H, m, *J*₃₋₄ 8.45, *J*₃₋₂ 6.6, *J*₃₋₁ 1, indazole), 8.3 (1H, m, *J*₁₋₂ 8.3, *J*₁₋₃ 1, indazole), 7.25 (2H, m, *o*-Ph), 7.3 (2H, m, *m*-Ph), 7.35 (1H, m, *p*-Ph); MS *m/z* (FAB) 333 (M⁺+1). MS *m/z* (EI) C₂₀H₁₆N₂O₃ requires 332.1161 found 332.1152.
- 16 The NOESY spectrum of the product which elutes first in the column shows space interactions between the signal at 5.9 ppm, (PhCH₂) and the signals at 6.65 ppm and at 7.2 ppm corresponding to the protons in the furyl ring i.e. the compound is *N*-2 alkylated **6**.
- 17 Biological assays were carried out using sGC purified from bovine lung (Alexis Inc) the activity was measured as described in S. J. Bunn, J. Garthwaite, and G. P. Wilkin, *Neurochem. Int.*, 1986, **8**, 179.