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A CONVENIENT ROUTE TO THE SOLUBLE GUANYLATE CYCLASE ACTIVATOR YC-1 AND ITS *N*2 REGIOISOMER

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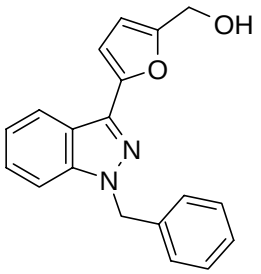
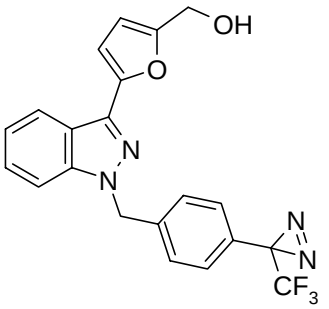
Abstract – A new route to the soluble guanylate cyclase (sGC) activator YC-1 and its *N*2 regioisomer has been established with a Mitsunobu mediated *N*-alkylation as the key step. The route is utilised in the synthesis of a potential photoaffinity label.

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]



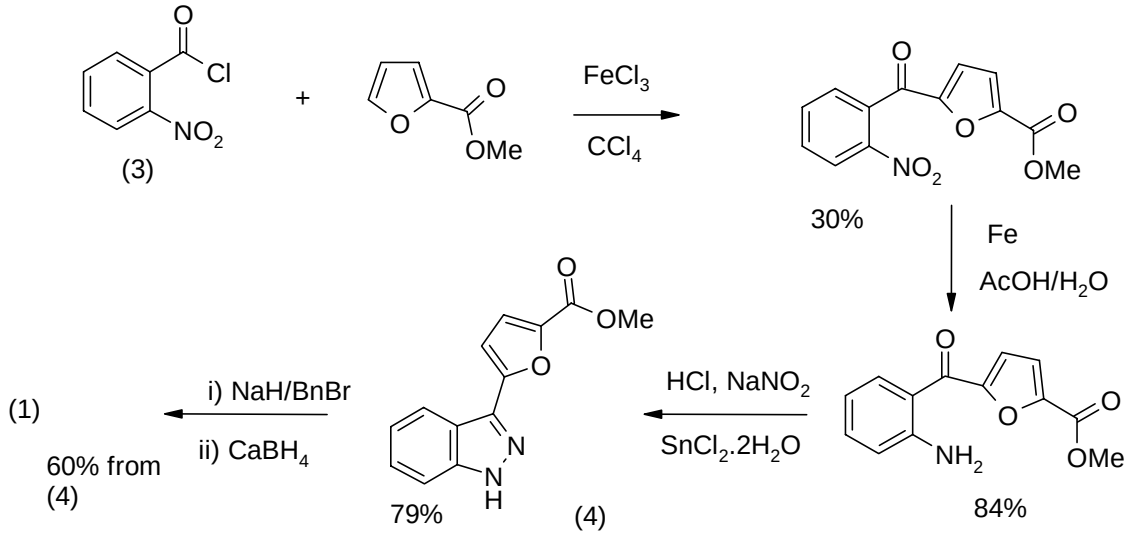
(1)



(2)

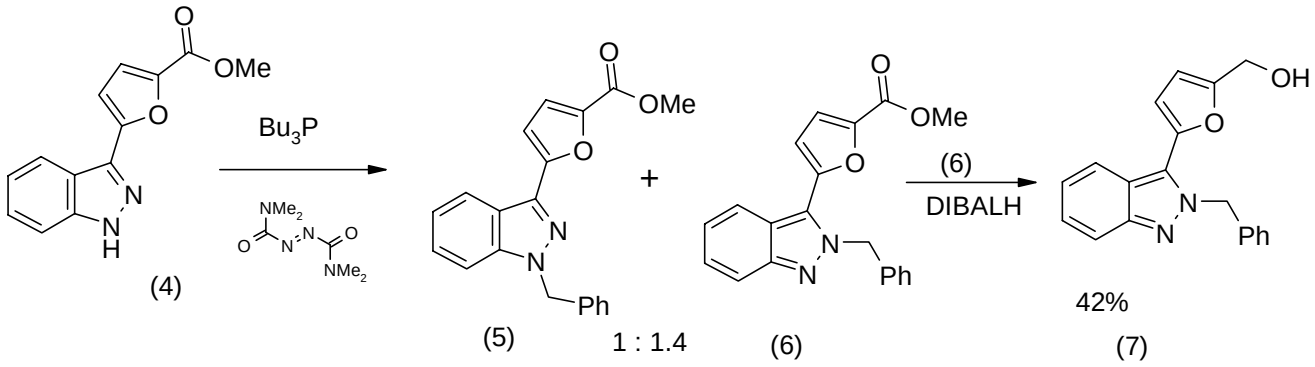
YC-1 (**1)** has been widely reported as an NO-independent activator of sGC,[4,5,6] though it is also known to be a non-specific inhibitor of phosphodiesterases[7] and to modulate the activity of Ca^{2+} -activated and voltage-dependent K^+ channels.[8] In connection with our studies[9] on novel activators 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 reagents [11] 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.



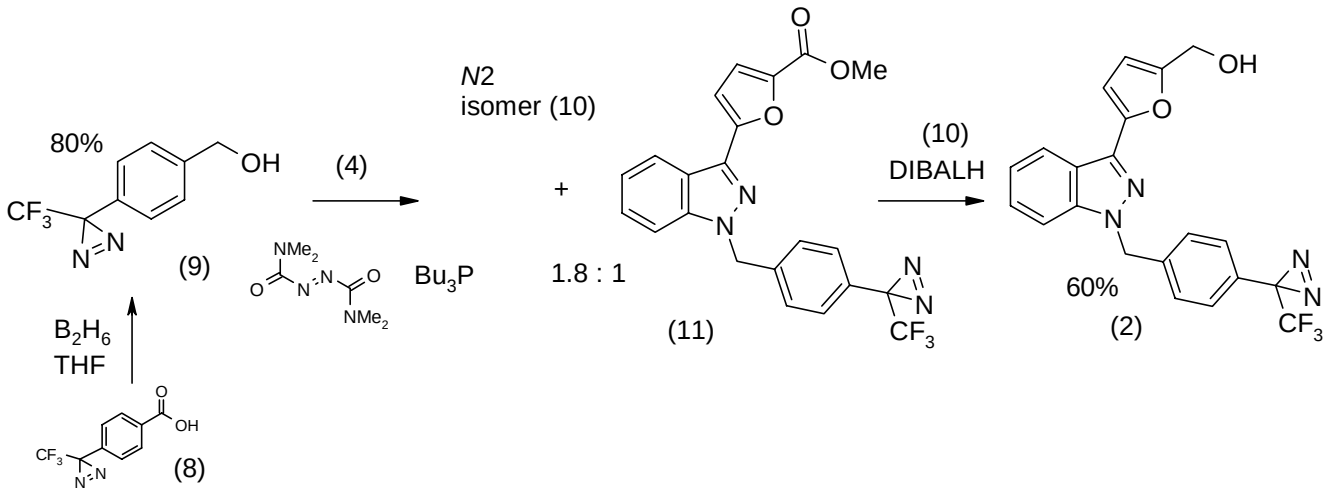
Scheme 1

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 conditions[9] would provide the required intermediate. To test the feasibility of the process we conducted the alkylation reaction under modified Tsunoda-Mitsunobu conditions[14] and obtained a mixture of *N*1 and *N*2 regioisomers[15] (**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 *N*2 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 $CaBH_4$ 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 *N*2 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 Bu_3P (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, (HYPERSIL, C18) to give **(6)** as a white solid (18.3 mg, 14 %), mp 112-120°C. δ_H ($CDCl_3$) 3.9 (3H, s, CO_2CH_3), 5.9 (2H, s, CH_2Ph), 6.75 (1H, d, *J* 3.5, furyl), 7.3 (1H, d, *J* 3.5, furyl), 7.3 (1H, m, $J_{2\text{-}1}$ 8.3, $J_{2\text{-}3}$ 6.6, $J_{2\text{-}4}$ 1.1, indazole), 7.8 (1H, dd, $J_{4\text{-}3}$ 8.45, $J_{4\text{-}2}$ 1.1, indazole), 7.4 (1H, m, $J_{3\text{-}4}$ 8.45, $J_{3\text{-}2}$ 6.6, $J_{3\text{-}1}$ 1, indazole), 8.3 (1H, m, $J_{1\text{-}2}$ 8.3, $J_{1\text{-}3}$ 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) $C_{20}H_{16}N_2O_3$ 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, ($PhC\underline{H}_2$) 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.