Selective Methylmagnesium Chloride Mediated Acetylations of Isosorbide: A Route to Powerful Nitric Oxide Donor Furoxans

Patrick Kielty,[†] Dennis A. Smith,[†] Peter Cannon,[‡] Michael P. Carty,[§] Michael Kennedy,[†] Patrick McArdle,[†] Richard J. Singer,[‖] and Fawaz Aldabbagh[*,†,⊥]

[†] School of Chemistry, National University of Ireland Galway, University Road, Galway, H91 TK33, Ireland

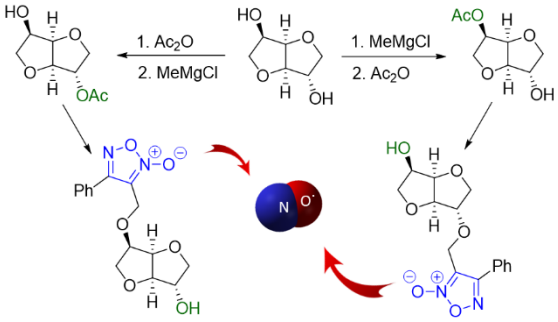
[‡] Avara Pharmaceutical Services, Shannon Industrial Estate, Shannon, Co. Clare, V14 FX09, Ireland

[§] Biochemistry, School of Natural Sciences, National University of Ireland Galway, University Road, Galway, H91 TK33, Ireland

[‖] Department of Chemical and Pharmaceutical Sciences, School of Life Sciences, Pharmacy & Chemistry, Kingston University, Penrhyn Road, Kingston upon Thames, KT1 2EE, UK

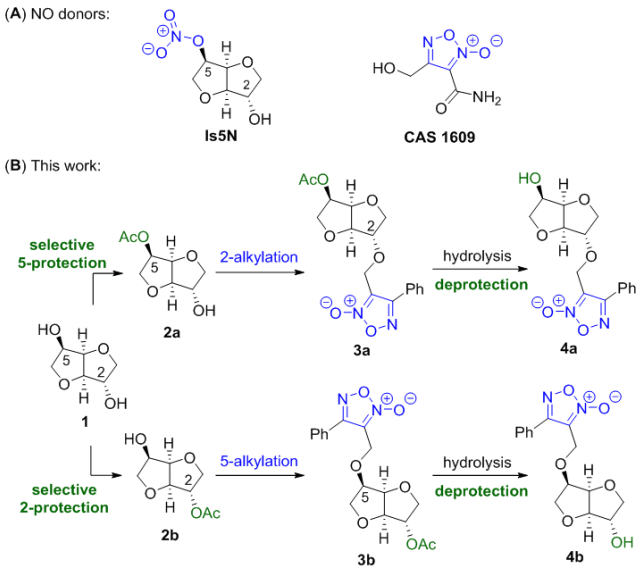
⊥ Present address: Department of Pharmacy, School of Life Sciences, Pharmacy & Chemistry, Kingston University, Penrhyn Road, Kingston upon Thames, KT1 2EE, UK

ABSTRACT: Isosorbide was functionalized with furoxan for the first time to give adducts that release nitric oxide up to 7.5 times faster than the commercial vasodilator, isosorbide-5-mononitrate (Is5N). The synthesis was facilitated by MeMgCl-mediated selective acetylation of isosorbide or selective deacetylation of isosorbide-2,5-diacetate, which was rationalised in terms of a more stable 5-alkoxide magnesium salt using DFT. Isosorbide-furoxans are safer to handle than Is5N due to greater thermal stability.



Nitric oxide (NO) is a reactive free radical with a vast array of physiological functions,[1] in cancer,[2] anti-microbial processes,[3] wound healing,[4] and most significantly, vasodilation.[5] Clinically, nitrate ester drugs are used to effect vasodilation. Nitroglycerin, pentaerythritol tetranitrate, isosorbide dinitrate (**IsDiN**), and isosorbide-5-mononitrate (**Is5N**, Scheme 1A) are prescribed to patients suffering from angina, where released NO induces relaxation of vascular smooth muscles (vasodilation) to reduce blood pressure.[6] Nitrate esters however, are well-known explosives,[7] including **IsDiN**, a particularly hazardous side-product in the manufacture of **Is5N**.[8] Furoxans (1,2,5-oxadiazole 2-oxides) are highly energetic molecules,[9] heralded as alternative NO-donors.[10-15] The Cassella-Hoechst drug, **CAS 1609**, is a furoxan with potent and long-lasting vasodilation,[10,11] and is devoid of the tolerance associated with nitrate ester drugs.[16] Isosorbide (**1**) is a sustainable feed-stock industrially produced from the double dehydration of D-sorbitol.[17] Apart from the nitrate esters, there are few valuable derivatives of **1** due to difficulties in selective functionalization and substitution at the 2- and 5-hydroxyl groups.[18] Herein, for the first time, furoxan is combined with isosorbide (**1**), and in doing so, we have developed a simple and selective acetylation protection-deprotection protocol for scaffold **1** (Scheme 1B).

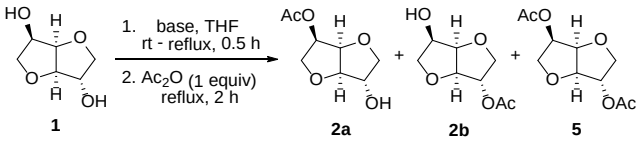
Scheme 1. (A) Is5N and furoxan drugs (B) Protection-deprotection of isosorbide 1 allowing selective functionalization with furoxan



The two hydroxyls of **1** are non-equivalent with an *exo* 2-OH and *endo* 5-OH having different reactivities. The more nucleophilic nature of the 5-OH is attributed to activation via H-bonding with the oxygen of the adjacent cycle.[19] Despite the difference in reactivity, base-mediated acetylations and alkylations are unreliable and low yielding, with the only reported selective acetylation of **1** using harmful PbO in Ac_2O to give isosorbide-5-acetate (**2a**).[20] Acetylation with Ac_2O alone gives mixtures of isosorbide acetates with 5-acetate **2a** the major product, but in low recovered yields.[21] The literature reported syntheses of isosorbide-2-acetate (**2b**) are inadequately selective with significant draw-backs. Preparations of **2b** have used DCC-mediated coupling with poor atom-economy,[22] and reduced-pressure distillation from mixtures of isosorbide-acetates.[20] More specialized enzyme-mediated acetylation of **1**, and enzyme-mediated hydrolysis of isosorbide-2,5-diacetate **5** have been reported.[23] Inexpensive MeMgCl has found application as a non-nucleophilic base, including in the Roche AG industrial deprotonation of secondary amines and α-deprotonation of nitriles.[24] Herein, the selective acetylation of the 2-OH and 5-OH of **1** was achieved by simply altering the number of equivalents of MeMgCl, as part of a protection-deprotection strategy that allowed facile alkylation to give unique furoxan NO-donors in high yields.

NaH and BuLi in combination with Ac_2O gave low conversions with little selectivity toward the 2- or 5-positions of isosorbide **1** (Table 1). MeMgCl (1.1 equiv) and Ac_2O provided high conversion and selectivity for acetylation at the 5-position, with 5-acetate **2a** isolated in 73% yield. By simply increasing the amount of MeMgCl (>2 equiv), a switch in selectivity of acetylation was achieved with the isomeric 2-acetate **2b** as the major product, occurring in a ratio of 45 : 1 over **2a**. Conversion was low, however, and replacing Ac_2O with more reactive AcCl enabled near complete conversion of **1** to **2b** in 78% isolated yield, with trace levels (<0.5%) of isomer **2a** detected by GC (Figure S2).

Table 1. Optimizing the formation of 2a and 2b

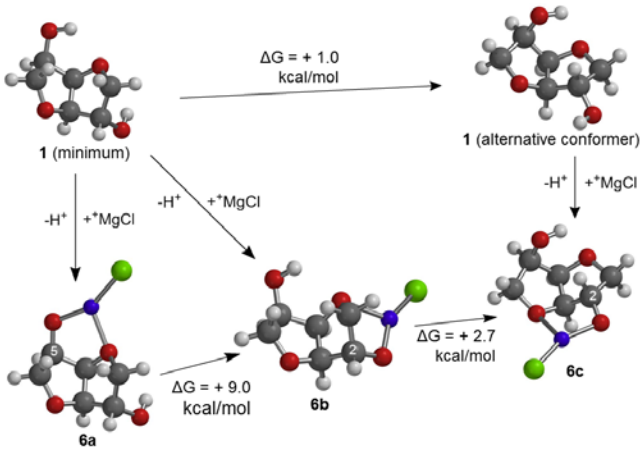


base (equiv)	**1** (%)[a]	**2a** (%)[b]	**2b** (%)[b]	**5** (%)[b]
NaH (1.1)	47	30	8	15
BuLi (1.1)	24	40	12	25
MeMgCl (1.1)	10	76 (73)[c]	2	13
MeMgCl (2.8)	43	1	45	11
MeMgCl (2.8)[d]	6	-	82 (78)[c]	12
MeMgCl (1.3)[e]	3	-	88 (83)[c]	9

See Table S1 for full list of optimization experiments. [a] Conversion determined by gravimetry. [b] Conversion determined by GC. [c] Isolated yield. [d] 0 °C, AcCl (1 equiv) as acetylation agent. [e] Ac_2O (2.5 equiv), Amberlite® IR-120, 2 h, 80 °C, then filtration, evaporation and addition of MeMgCl, THF, reflux, 8 h.

DFT modelling was used to investigate this remarkable selectivity of deprotonation by assessing the stability of $^{+}$MgCl complex **6a** relative to **6c** (Scheme 2) formed through ethereal (THF-like)[25] chelation to the adjacent ring oxygen atom in the isosorbide substrate. DFT was carried out in the gas phase, since solvent effects (the role of THF) would not affect the relative energies of the observed isosorbide complexes. Two conformations of **1** were of interest; the minimum energy model and an alternative conformer 1.0 kcal/mol higher in energy. Starting from the minimum energy conformer **1**, coordination of the $^{+}$MgCl ion was preferred at the 5-alkoxide **6a** by 11.7 kcal/mol over the strained 2-alkoxide **6c**. The greater stability of **6a** arises from more effective $^{+}$MgCl coordination to the oxygen atom on the adjacent ring with steric hindrance from the isosorbide junction 3,4-hydrogens affecting coordination in complex **6c**. The energy minimum conformation of **1** preferentially gave 2-alkoxide complex **6b**, with ethereal $^{+}$MgCl coordination within the same ring, over 2-alkoxide **6c** by 2.7 kcal/mol. Moreover, $^{+}$MgCl coordination to 2-alkoxide **6c** was only possible using the higher energy alternative conformer of **1**. The enhanced stability of 5-alkoxide **6a** over 2-alkoxide complexes **6b** and **6c** was reflected in shorter $Mg^{+}\cdots^{-}OR$ (alkoxide) and $Mg^{+}\cdots OR_2$(ether) bond distances (Table S3).

Scheme 2. DFT of proposed alkoxide intermediates from the reaction of isosorbide 1 with MeMgCl [a]



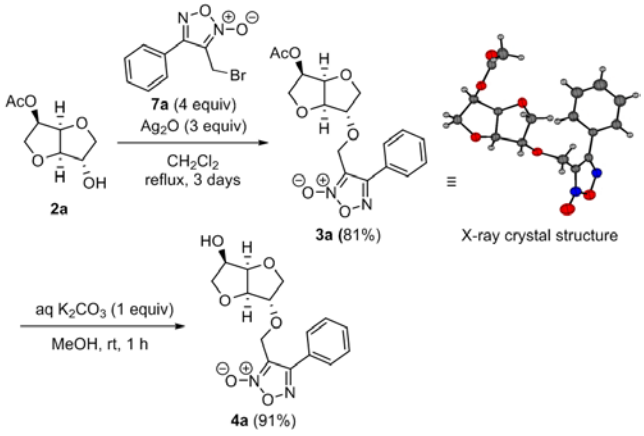
[a] All geometry optimizations were performed using Gaussian 16W/GaussView 6 with DFT B3LYP functional in the gas phase and a 6311G (2d,p) basis set.[26] Mg and Cl atoms depicted in blue and green, respectively. Table S2 contains model energies in Hartrees.

Upon addition of >2 equiv of MeMgCl, both the 2- and 5-OHs of **1** were deprotonated, and acetylation occurred at the less stable, more reactive 2-alkoxide, delivering a switch in selectivity to give **2b**. DFT modelling supported the greater stabilization of $^{+}$MgCl at the 5-alkoxide in the 2,5-dialkoxide complex with shorter bond lengths between $Mg^{+}\cdots^{-}OR$(alkoxide) and $Mg^{+}\cdots OR_2$(ether) compared with $^{+}$MgCl coordination at the 2-position (Figure S3). Using the principle of forming the more thermodynamically stable $^{+}$MgCl intermediate (i.e. the 5-alkoxide, analogous to **6a**), a selective mono-deacetylation of isosorbide-2,5-diacetate **5**

with MeMgCl (1.3 equiv) was carried out (Table 1). The multi-gram synthesis of isosorbide-2-acetate (**2b**) was achieved in 83% yield without the requirement for chromatography with diacetate **5** formed *in situ* from **1** using Ac_2O and Amberlite® IR-120 catalyst.

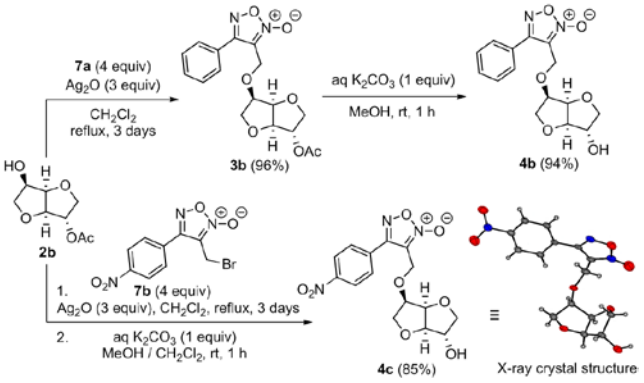
With the 2- or 5-positions on isosorbide effectively blocked through acetylation, functionalization of the available hydroxyl with furoxan was now possible. Furoxan electrophiles **7a** and **7b** can be readily prepared in two steps from their respective cinnamyl alcohols (see Supporting Information).[14,15] Alkylation with the furoxan bromides **7a** and **7b** was mediated by Ag_2O. Reaction of 5-acetate **2a** with bromide **7a** yielded isosorbide-5-acetate-2-furoxan **3a** in 81% yield with furoxan attachment at the *exo*-position confirmed by X-ray crystallography (Scheme 3).

Scheme 3. Synthesis of isosorbide-2-furoxan 4a and X-ray crystal structure of 3a (thermal ellipsoids set at 40% probability)



Deprotection of 5-acetate **3a** through basic hydrolysis afforded isosorbide-2-furoxan **4a** in 91% yield. Similarly, synthesis of furoxan isomer **4b** from 2-acetate **2b** via isosorbide-2-acetate-5-furoxan **3b** occurred in an overall 90% yield over two steps of alkylation and deprotection (Scheme 4).

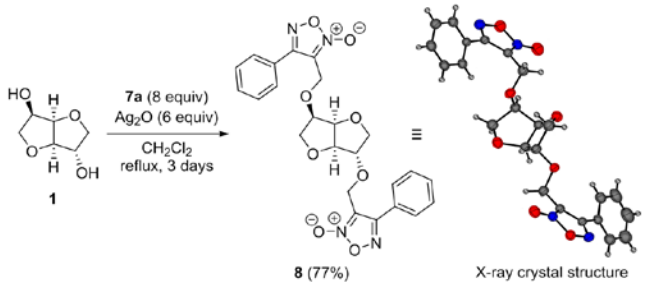
Scheme 4. Synthesis of isosorbide-5-furoxans 4b and 4c and X-ray crystal structure depicting one of the two molecules in the asymmetric unit cell of 4c (thermal ellipsoids set at 50% probability)



It is known that NO–production can be increased by substituting the furoxan ring with electron-withdrawing groups, such as nitrobenzene, which effectively increase susceptibility to addition of activating thiols (such as cysteine).[13,14] Thus, in a quest to increase the activity of isosorbide-5-furoxan, the preparation of *p*-nitro derivative **4c** was carried out from isosorbide-2-acetate **2b** and bromide **7b** (Scheme 4). *p*-Nitrophenyl-substituted furoxan **4c** was isolated in 85% yield without the requirement for isolation of the intermediate isosorbide-2-acetate-5-furoxan.

To further increase the level of NO-release, isosorbide was functionalized with two furoxan moieties. The preparation of isosorbide-2,5-difuroxan **8** was achieved in 77% isolated yield by facile alkylation of **1** with **7a** in the presence of Ag_2O (Scheme 5). The X-ray crystal structure of bis-adduct **8** was obtained, and like the other two crystal structures (of **3a** and **4c**) provided a clear visual representation of the 2-*exo* and 5-*endo* isosorbide attachments (Schemes 3-5). H-bonding in the lattice only occurred in **4c**, presumably due to the isosorbide hydroxyl being deacetylated, (Fig. S10 and Table S5B).

Scheme 5. Synthesis and X-ray crystal structure of isosorbide-2,5-difuroxan 8 (thermal ellipsoids set at 30% probability)



NO-release from our isosorbide furoxans was quantitatively measured along with that of the commercial vasodilator, **Is5N**. Furoxans have long been known to release NO upon reaction with thiols, including cysteine.[12] DFT calculations supported the addition of a sulfanyl radical onto the C=N bond of the furoxan followed by expulsion of NO,[27] although others give alternative thiolate addition mechanisms,[12,14] which are energetically less favourable.[27] NO-release holds a reasonable correlation with vascular tissue relaxation in a series of furoxans.[11,12] NO is, however, never measured directly due to very rapid oxidation in aqueous and biological fluids to nitrite (NO_2^-) and nitrate (NO_3^-).[12,14] The amount of NO-released is commonly evaluated through NO_2^- measurement using the Griess assay, in the presence of excess cysteine with the presence of NO_3^- deemed less significant. Using this approach, NO_2^- production from isosorbide-furoxans **3a**, **4a**-**4c** and **8** was quantitatively monitored by regular sampling (Figure S4), which indicated a 3-7.5 fold greater rate of release than **Is5N** (k_{obs}, Table 2). After 12 h a significant slowdown for most furoxans was observed, apart from isosorbide-2,5-difuroxan **8**, which exhibited a longer sustained NO-release. This retardation in NO release allowed measurement of the extent of NO_2^- production after 24 h (Table 2). A thorough approach was used with combined NO_2^- and NO_3^- measured.

Nowadays this is possible using a commercially available colorimetric assay that measures total available NO_2^- after reduction of NO_3^- to NO_2^- by NADPH in the presence of the enzyme, nitrate reductase.[28] Moreover, Table 2 shows NO_3^- levels were significant for some furoxans, especially the most reactive compounds highlighting the need for analysis.

Table 2. Extent and rate of NO release

NO donor	NO_2^- produced (mol %) [a]	NO_2^- + NO_3^- produced (mol %) [b]	k_{obs} (h^{-1}) [c]
3a	43.8 ± 1.7	45.2 ± 1.6	0.081
4a	56.0 ± 1.0	61.7 ± 3.5	0.098
4b	47.3 ± 1.4	47.4 ± 0.7	0.079
4c	60.9 ± 1.8	67.5 ± 1.6	0.195
8	80.3 ± 1.1	94.5 ± 4.0	0.136
Is5N	19.0 ± 0.6	19.0 ± 1.2	0.026

[a] Determined by Griess assay after 24 h incubation of 2.0 mM solutions with excess L-cysteine at 37 °C. [b] Samples of solutions after 24 h incubated with nitrate reductase prior to performing Griess assay. [c] Rate constant (k_{obs}) for NO_2^- production calculated by linear regression analysis of 0-4 h data points for **4c** and 0-8 h data points for all other compounds in Figure S4.

The *p*-nitrophenyl-substituted derivative furoxan **4c** had a significantly higher rate of NO-release, with this compound being the most active, with 2-2.5 times greater rate of NO_2^- production compared to isosorbide-2- and 5-furoxans **4a** and **4b**. High concentrations of NO have been related to anti-cancer activity for some furoxans.[29] The acetate group of **3a** appeared to hinder NO-release with a slower rate, as well as a smaller total amount of NO produced compared to its hydrolyzed derivative **4a**. As expected, substituting both available hydroxyls on isosorbide with furoxan in compound **8** almost doubled the extent of NO production, but the rate of NO-release was below that of the most reactive mono-substituted isosorbide furoxan **4c**. Isosorbide-2-furoxan **4a** gave higher levels of NO-release compared to its isomer **4b**, and pharmacologically they would be expected to behave differently as shown by the drug **Is5N**, which is less potent than isosorbide-2-mononitrate (**Is2N**), as a vasodilator.[30]

Differential Scanning Calorimetry (DSC) was used for assessment of thermally induced energy release,[31] which allows deductions regarding safe handling in comparison with **Is5N**, which is classified as an explosive.[32] The onset temperature for thermal degradation of phenyl furoxans (**4a**, **4b** and **8**) is less accessible at 66-94 °C above that of **Is5N** (Table 3). Similarly, the degradation exotherms peaked 61-73 °C above that of **Is5N**. The energy released from such exothermic events was lower for the phenyl furoxans by 1086-1614 J/g. The most active NO-releasing furoxan, *p*-nitrophenyl furoxan **4c** is safer than **Is5N** with a significantly higher onset and peak temperature by almost 32 °C and 41 °C respectively, and with an energy release 1099 J/g lower.

In summary, inexpensive MeMgCl-mediated acetylation has afforded an effective and simple protection-deprotection for both hydroxyls of isosorbide. Subsequent functionalization with furoxan gave powerful nitric oxide donors with vastly higher rates and amounts of NO_X produced compared to the commercial vasodilator, **Is5N**. Isosorbide-furoxans are safer to handle than **Is5N**. Future studies will focus on the biological evaluation of this new class of NO-donors.

Table 3. Exothermic events [a]

NO donor	onset temp (°C) [b]	peak temp (°C) [c]	energy release (J/g) [d]
4a	231.0	271.8	1122.0
4b	202.9	261.4	1474.5
4c	168.3	241.7	1636.9
8	217.9	273.7	1649.5
Is5N	136.7	200.4	2735.8

[a] DSC was performed using 1.7-4.6 mg of samples in a high pressure crucible, heating at 5 °C/min. [b] Temperature at which the exothermic event begins. [c] Temperature of maximal heat release. [d] Calculated by integration.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental, synthetic procedures, characterization data, NMR spectra, DFT, DSC and crystallographic data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

CCD 1830485, 1830483 and 1830484 contain the supplementary data for compounds **3a**, **4c** and **8** respectively. This data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

AUTHOR INFORMATION

Corresponding Author

*E-mail: f.aldabbagh@kingston.ac.uk

ORCID

Fawaz Aldabbagh: 0000-0001-8356-5258

Notes

The authors declare no competing financial interest.

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