Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supplementary Information for

Studies of nitrile oxide cycloadditions, and the phenolic oxidative coupling of vanillin aldoxime by *Geobacillus sp.* DDS012 from Italian Rye Grass silage

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Abstract

During studies directed towards the discovery of nitrile hydrolysing enzymes from thermophiles, vanillin aldoxime was incubated with the thermophilic organism, *Geobacillus sp.* DDS012 isolated from Italian Rye Grass (*Lolium multiflorum*) silage. The predominant product was a dihydro-dimer, which could only be characterised by LC-MS. This was initially imagined to be the product of cycloaddition of vanillin aldoxime with the corresponding nitrile oxide, but preparation of the supposed adduct and model studies excluded this possibility. The rate constant for the second order dimerisation of 4-*O*-acetyl vanillin nitrile oxide was measured (1.21 x 10^{-4} M^{-1} s^{-1}, 0.413 molar, 25 °C) and the ^{13}C-NMR signal for the nitrile oxide carbon was observed (δ_C 34.4, *br. t* ^1J ^{13}C,^{14}N circa 50 Hz). Treatment of vanillin aldoxime with potassium persulphate and iron sulphate gave material with the same LC-MS properties as the natural product, which is therefore identified as 5,5´-dehydro-di-(vanillin aldoxime) **1d** formed by phenolic oxidative coupling.

This supplementary material uses references with the same numbering system as the published paper. The following references are first used in the published paper.
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36, 37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,5253,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69, 70,71,72,73,74,75,76,77,78,79,80,81,82,83

Experimental

Microbiology Experimental

Full details of the activity of other isolates, carbohydrate assimilation studies and genetic analysis will be published elsewhere. All incubations were carried at 70 °C, in an orbital shaker at 150 rev min^{-1} for 24 hrs, unless noted otherwise

HPLC was carried out using a Agilent series HPLC (Brucker Daltonics, Coventry, UK) fitted with a 3 cm x 4.6 cm Nucleosil ODS 5 μm guard column, a 25 cm x 4.6 cm, C18 Nucleosil ODS 5 μm column (Jones chromatography) and a UV detector (254 nm). The column was eluted with aqueous orthophosphoric acid (0.2 %):acetonitrile (70:30) at 1 ml/min,.

Fresh samples of Italian Rye Grass (*Lolium multiflorum*) silage were collected from an unopened eight week old silo at a dairy farm located in Beckington, Somerset, UK into sterile glass bottles and refrigerated at 4 (± 2) °C until further use. Silage (5 g) was added to sterile media (200 ml): *Thermus* 878[84], Castenholz[85] or Luria-Bertani media, or nutrient broth (Difco Labs.) and incubated on an orbital shaker at 70 °C, 150 rev min^{-1} for 24 hours. Cell density was measured using the absorbance at 600 nm. The silage enrichment cultures were incubated and serially diluted with an equal volume of media, every 7 days for up to two months. Aliquots from these enrichment cultures were serially diluted and streaked onto solid versions of the liquid media to isolate single colonies.

Cultures were stored for a few days at 4 °C on agar plates. For longer term storage, the liquid culture was centrifuged (9600 x g, 10 mins.) and the supernatant was discarded. The pellet was washed with phosphate buffer (100 mM, pH 7.0, 25 ml) and resuspended in glycerol (20 μl) and *Thermus* 878 medium (80 μl) and stored at -80 °C.

Characterisation of DDS012

Differential biochemical characteristics.

DDS012; production of acid from: adonitol (-); L-arabinose (-); cellobiose (-); fructose (+); galactose (-); glucose (+); glycerol (+); inositol (-); lactose (-); maltose (+); mannitol (-); mannose (+); L-rhamnose (-); ribose (-); sorbitol (-); sucrose (+); trehalose (+); D-xylose (-). starch hydrolysis (+); utilisation (Simmons) of citrate (-).

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G. Thermodenitrificans; ND = not determined; production of acid from: adonitol ND; L-arabinose (+); cellobiose (+); fructose (+); galactose (+); glucose (+); glycerol (+); inositol ND; lactose (+); maltose (+); mannitol (+); mannose (+); L-rhamnose, variable; ribose (+); sorbitol ND; sucrose (+); trehalose (+); D-xylose (+). starch hydrolysis, variable; utilisation (Simmons) of citrate, variable.

Carbohydrate assimilation profile All strains were identified by using carbohydrate assimilation and fermentation of 49 different carbohydrates (and one positive control) using API 50 CHB^{86} strips (bioMérieux, France). The strips were incubated at 70° C for 24 hrs and the colour change checked at 3, 6 and 24 hrs. The organisms were identified using APILAB Plus software version 3.3.3 from the bioMérieux computer database. DDS012, *Bacillus firmus* (ID % 94.4, T-index 0.61).

16S rDNA DDS012, *Geobacillus thermodenitrificans* OHT-1 (97 % sequence similarity) Strain names, GenBank accession numbers: DDS001, EF426757; DDS003, EF426758; DDS005, EF426759; DDS006, EF426760; DDS010, EF426761; **DDS012, EF426762**; DDS013, EF426763; DDS014, EF426764; DDS015, EF426765; DDS016, EF426766; DDS017, EF426767; DDS018, EF426768; DDS019, EF426769; DDS021, EF426770; DDS022, EF426756.

Biotransformation growth curves A colony (from a freshly grown culture plate) was picked and inoculated under aseptic conditions into nutrient broth (10 ml) and incubated. A stock growth media was prepared from "nitrogen free" *Thermus* 878 medium and succinic acid (final concentration 25 mM) and the required amount of vanillin aldoxime **1b** (5 mM final concentration). Stock culture media (50 ml) was added to a conical flask under aseptic conditions and an aliquot of freshly grown cells were added until the final optical density (600 nm) of the culture reached ~0.02. The culture was incubated and the increase in optical density was measured over 24 - 36 hours.

Induction Strain DDS012 was cultured in *Thermus* medium 878 at 70 °C for 12 hrs at 150 rev. min^{-1} in an orbital shaker. Once the cultures reached an optical density of ~0.6 (measured at 600 nm) the cells were harvested and washed twice with phosphate buffer (25 ml, pH 7.2). The cells were resuspended and cultured in "nitrogen free" *Thermus* 878 medium (50 ml) containing sodium succinate (25 mM) and vanillin aldoxime **1b** (5 mM). Each experimental flask (contained 50 ml of the medium in a 250 ml flask) contained a 2 % inoculum from the starter culture. This culture was incubated for a further 8 - 12 hrs. The growth was monitored by removing aliquots of medium (1 ml) and by measuring the optical density at 600 nm. The cells were harvested at the mid exponential phase

of growth (OD_{600} 0.4-0.8) by centrifugation (15,000 g; 10 min.; room temperature), washed twice with phosphate buffer (100 mM, pH 7.4) and resuspended in fresh buffer to give 4.5 - 5.0 mg/ml of cells.

Biotransformation of aldoximes by substrate induced cultures Vanillin aldoxime **1b** (5 mM) in 50 % aqueous ethanol was added to the cell suspension in phosphate buffer (100 mM, pH 7.4) and the biotransformation was carried at 70 °C in an orbital shaker at 150 rev min^{-1}. Aliquots (500 µl) of the reaction mixture were removed every hour and cells removed by centrifugation (13,000 g; 5 min.; 4 °C). The cell pellet was discarded and the supernatants were collected and stored at -20 °C before analysis. All experiments were carried out in triplicates and the mean activity was calculated. HPLC showed the disappearance vanillin aldoxime **1b** (RT 5.52) and the formation of of trace amounts of vanillamide **3b** (RT 4.81 min), vanillic acid **4b** (RT 5.06 min), vanillin **5b** (RT 7.83 min) and vanillin nitrile **2b** (RT 8.92 min). These were identified by comparing the retention times with authentic standards and subsequently by HPLC-MS. The major product was vanillin aldoxime dehydro-dimer **1d** (RT 11.0 min). The degradation of VOX was significantly ($P < 0.01$) higher at 48 hours for the strains DDS010, DDS012, DDS013, DDS018, DDS023 and DDS026 compared to the rest of the strains. After 55 to 60 hrs, neither vanillin aldoxime **1b** or the products were detected by HPLC analysis.

HPLC for MS

Separations were carried out using a Varian Model 9012 HPLC pump (Varian, Walton-on-Thames, Surrey, UK) and a reversed-phase 3 µm Aquastar HPLC column 100 x 2.1 mm (Thermo-Electron, UK). Samples were dissolved in methanol (1 mg/mL) and diluted 1:1 with the initial eluent. 0.1 µL injections were made using a Valco microinjector (Valco, Houston, TX, USA) and eluted with the following gradient (Table 1).

Table 1, HPLC solvent gradient for HPLC-MS

Time (mins.)	0	15	20
95 % Water / 5 % CH_3CN + 0.2 % formic acid	100	25	5
CH_3CN + 0.2 % formic acid	0	75	95

Mass Spectrometry.

Data were acquired on a Micromass Q-Tof *micro* Electrospray Ionisation (ESI), orthogonal acceleration quadrupole / time-of-flight mass spectrometer (Micromass, Waters, Manchester, UK). Data was acquired in positive mode with the probe voltage set at 3.5 kV and a cone voltage of 30 V. The source and desolvation temperatures were set to 80 and 110°C (for syringe infusion) and 110 and 300°C (for HPLC), respectively. Nitrogen desolvation and nebulizer gas flows were 350 and 50 L/h,

respectively. The instrument was calibrated over the range *m/z* 100-1000 using a mixture of polyethylene glycol (PEG), 300, 400 and 600, 1, 1, 2 ng/μL respectively, dissolved in 50:50 v/v acetonitrile / 10 mM aqueous ammonium acetate. This was infused by the in-built instrument syringe-driver at a rate of 5 μL/min. Spectra were acquired in continuum mode at a rate of one spectrum/3 s. MS/MS was carried out using Argon as collision gas with collision energy of 20 eV.

For accurate mass measurements, a mixture of PEG, as used for the initial instrument calibration was added to the analyte solution and infused from the syringe-driver as before. One of the PEG series ions at *m/z* 327 was used as an internal "lock-mass" to adjust the instrument *m/z* calibration and so provide accurate mass measurement for the analyte. The ratios of the analyte ion and PEG ion were kept at an approximate ratio of 1:1, by adjustment of relative concentrations and ion counts per second, below 200.

Table 2. HPLC-QTOF-MS (ESI+) data for vanillin aldoxime dimers

Analyte (HRMS)	*m/z* of major ion peaks (%) for peaks with retention time 14.28 mins
Natural **1d** (333.1081)	333.1 (M + H; 48); 316.1 (62); 297.1 (100); 283.1 (55); 270.1 (45); 266.1 (30); 253.1 (55); 240.1 (20); 273.1 (35)
Synthetic **1d** (333.1086)	333.1 (M + H; 60); 316.1 (50); 297.1 (100); 283.1 (70); 270.1 (40); 266.1 (30); 253.1 (44); 240.1 (19)
"Heterocycle" **12b**	(20 eV) 168.1 (100); 163.1 (38); 153.1 (22); 151 (17); 149.1 (31); 135.1 (82); 125.1 (20) (10 eV) 333.2 (M + H; 25); 181.1 (18); 168.1 (63); 163.1 (100); 153.1 (37); 135.1 (33)

Composition analysis of accurate mass spectrometry data for *6,6´-dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dialdoxime (vanillin aldoxime dehydro-dimer)* **1d** ("natural") Found 333.1081, calculated (error ppm) $C_{16}H_{17}N_2O_6$, 333.1087 (-1.7); $C_{15}H_{11}N_9O$, 333.1086 (-1.5); $C_{14}H_{15}N_5O_5$, 333.1073 (+2.3); $C_{17}H_{13}N_6O_2$, 333.1100 (-5.7); $C_{13}H_{19}NO_9$, 333.1059 (+6.5); $C_{12}H_{13}N_8O_4$, 333.1059 (+6.5); $C_{19}H_{15}N_3O_3$, 333.1113 (-9.7).

Chemical Experimental

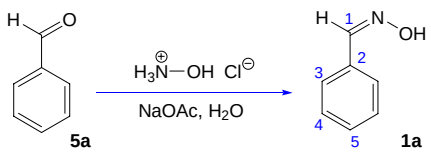
Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Columns were eluted with a gradient starting with a low polarity solvent and then increasing amounts of a more polar solvent. Analytical thin layer chromatography was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF_{254}, which were visualised under UV light (at 254 and/or 360 nm) and stained using an ethanolic phosphomolybdic acid dip (3 %) and heating. All products were homogenous as judged by TLC unless stated otherwise.

Low resolution mass spectra were obtained using a Fisons VG Platform II Quadrupole spectrometer with electron impact (EI; 70 eV), atmospheric pressure chemical (ApcI) or electrospray (ESI)

ionisation. High resolution mass spectra were obtained at the EPSRC mass spectrometry service, Swansea. Melting points (mpt.) were determined on a Kofler hot stage apparatus and are uncorrected. Infrared spectra were recorded in the range 4000–600 cm^{-1} on a Perkin-Elmer 1600 series FTIR spectrometer using KBr disks for solid samples and thin films between NaCl plates for liquid samples and are reported in cm^{-1}. Signals are annotated using the usual conventions (str. strong; w. weak; sh. sharp; br. broad).

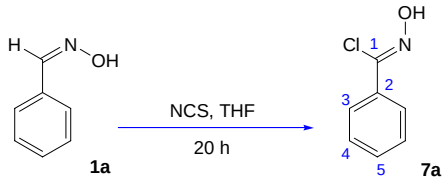
^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on Bruker Avance 500, Bruker DPX-400, or Bruker DPX-250 spectrometers, at 500, 400 and 250 MHz for ^{1}H spectra and at 125, 100 or 62.5 MHz for ^{13}C spectra. For spectra recorded in $CDCl_3$, chemical shifts are measured relative to tetramethylsilane (δ 0.00 ppm) or to the resonances of residual $CHCl_3$ (δ 7.28 ppm for ^{1}H and δ 77.0 ppm (the central line of the triplet) for ^{13}C. *J* values are recorded in Hz and multiplicities are expressed by the usual conventions (*s* singlet; *d* doublet; *t* triplet; *app* apparent; *br.* broad; *m* multiplet). Coupling constants were determined using the computer program Multiplet (release NMRUC51, D. R. Kelly, unpublished work). Multiplet, uses peak positions from peak listings to calculate line spacings which are aggregated (± 0.5 hz) and averaged to give putative couplings. These in turn are permutated to give possible coupling patterns. Thus the calculated coupling constants have an accuracy which is only limited by the digital resolution of the NMR machine and line broadening effects. Values are reported to 0.1 Hz, but have an uncertainty of =< 0.4 Hz (at 400 MHz, 15 ppm for routine ^{1}H-spectra), due to the digital resolution of the FID accumulation and Fourier transformation. ^{1}H-NMR spectra were simulated using RACCOON (University of Wisconsin). All compounds were analysed using ^{1}H, ^{13}C-DEPT, *J*-^{1}H,^{1}H-COSY, ^{1}J-^{1}H,^{13}C- and ^{3}J-^{1}H,^{13}C-COSY experiments plus in some cases APT. All assignments were made using correlations based on signals of unambiguous chemical shift (eg. ^{13}C C=O, or ^{1}H- CH_3O). In the few cases where this was not possible ^{1}H and ^{13}C-NMR chemical shifts were predicted using the routines in ChemDraw 8.0 or using other substituent increment rules, these assignments are annotated as csp (chemical shift prediction).[87]

Preparation of phenyl derivatives

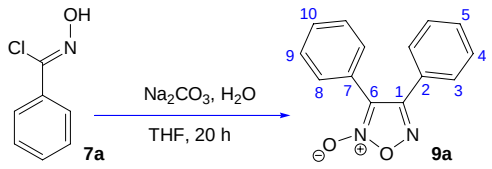


(Z)-benzaldoxime **1a** A solution of hydroxylamine hydrochloride (34.7 g, 0.5 mol) in water (150 ml) and a solution of sodium acetate (50 g, 0.61 mol) in water (150 ml) were added to a solution of benzaldehyde **5a** (50 g, 0.47 mol) dissolved in water (240 ml). The reaction was stirred at room

temperature for 24 h and extracted three times with dichloromethane (150 ml, 2 x 75 ml). The combined organic extracts were washed with saturated sodium hydrogen carbonate solution (3 x 50 ml) and water (50 ml), dried with anhydrous sodium sulphate and the solvent evaporated, to give *(Z)-benzaldoxime* **1a** as a viscous colourless liquid (56.3 g, 99 %, lit. mpt. 30 °C[50]), which was used without further purification. δ_H ($CDCl_3$) circa 14.0 (1H, v. br., OH); 7.44 (3H, m, 4-H_2, 5-H); 7.63 (2H, m, 3-H_2); 8.21 (1H, s, 1-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 127.1 (CH, 7.63, 3-C); 128.8 (CH, 7.44, 4-C); 130.1 (CH, 7.44, 5-C); 131.9 (C, 2-C); 150.4 (CH, 8.21, 1-C); ν_{max} (neat) 3311, 3063, 2895, 1632, 1601, 1493, 1445, 1304, 1210, 951, 870, 755, 691.

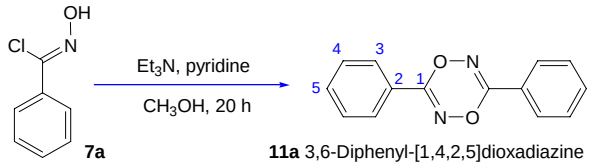


(Z)-Benzaldehyde oximyl chloride **7a** (*Z*)-Benzaldoxime **1a** (2 g, 16.5 mmol) was dissolved in tetrahydrofuran (10 ml) and added to a solution of *N*-chlorosuccinimide (2.24 g, 16.8 mmol) in tetrahydrofuran (10 ml). After 20 h dichloromethane (50 ml) was added. The resulting solution was washed with hydrochloric acid (1 M, 2 x 20 ml) and water (2 x 20 ml), dried with anhydrous sodium sulphate and evaporated to give benzaldehyde oximyl chloride **7a** (2.1 g, 82 %) as a colourless viscous liquid, which could be stored below 0 °C for several weeks without decomposition. *Benzaldehyde oximyl chloride* **7a** δ_H ($CDCl_3$) 7.46 (3H, m, 4-H_2, 5-H); 7.88 (2H, m, 3-H_2); 8.76 (1H, br. s, OH); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 127.2 (CH, 7.88, 3-C); 128.5 (CH, 7.46, 4-C); 130.7 (CH, 7.46, 5-C); 132.5 (C, 2-C); 139.9 (C, 1-C); δ_H (C_6D_6) 7.10 (3H, m, 4-H_2, 5-H); 7.93 (2H, m, 3-H_2); 8.08 (1H, br. s, OH); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 127.2 (CH, 7.93, 3-C); 128.4 (CH, 7.10, 4-C); 130.3 (CH, 7.10, 5-C); 133.0 (C, 2-C); 139.0 (C, 1-C); ν_{max} (neat) 3246 (str. br.); 2980 (str.); 2877 (str.); 1705; 1602; 1578; 1493; 1446 (str.); 1235 (str.); 1049; 998.



3,4-Diphenyl-1,2,5-oxadiazol-2-oxide **9a** A solution of saturated sodium carbonate (5 ml) was added to a solution of benzaldehyde oximyl chloride **7a** (0.26 g, 1.67 mmol) in diethyl ether (10 ml). The mixture was stirred rapidly for 24 h and then transferred to a separating funnel. The organic layer was removed and the aqueous layer extracted with diethyl ether (2 x 10 ml). The combined organic layers

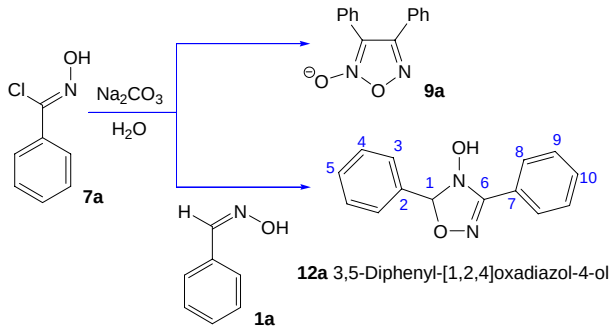
were washed with water (2 x 5 ml) and dried with anhydrous magnesium sulphate. Evaporation of the solvent yielded crude 3,4-diphenyl-1,2,5-oxadiazol-2-oxide **9a** (0.13 g, 65 %). Recrystallisation from ethanol gave white needles (0.06 g, 30 %). *3,4-diphenyl-1,2,5-oxadiazol-2-oxide* **9a:** δ_H ($CDCl_3$) 7.40 (5H, m, 4-H_2, 9-H_2, 10-H); 7.46 (5H, m, 3-H_2, 5-H, 8-H_2); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 114.3 (C, 6-C); 122.9 (C, 7-C); 126.8 (C, 2-C); 128.3 (CH, 7.46, 8-C); 128.7 (CH, 7.46, 3-C); 129.0 (CH, 7.40, 4-C or 9-C); 129.1 (CH, 7.40, 4-C or 9-C); 130.6 (CH, 7.40, 10-C); 131.0 (CH, 7.46, 5-C); 156.3 (C, 1-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 114.3 (7.46, 6-C); 122.9 (7.40, 7-C); 126.8 (7.40, 2-C); 128.3 (7.40, 8-C); 128.7 (7.46, 3-C); 129.0 (7.40, 4-C or 9-C); 129.1 (7.40, 4-C or 9-C); 130.6 (7.46, 10-C); 131.0 (7.46, 5-C); 156.3 (7.46, 1-C); δ_H (DMSO-d_6) 7.58 (10H, br. m, bandwidth circa 67 Hz); δ_C 114.5* (C, 6-C); 122.7* (C. 7-C); 126.3* (C, 2-C); 128.1 (CH); 128.9 (2 x CH); 129.0 (CH); 130.6* (CH, 10-C); 131.1* (CH. 5-C); 156.6* (C, 1-C); * = identical to those reported previously; ν_{max} (KBr) 1591[46], 1504, 1474, 1453, 1442, 1421, 772, 692, 655; Mpt. 109-111 °C (lit. mpt. 108 - 110°C[88], others have reported up to 117 - 118°C[46]).



7a **11a** 3,6-Diphenyl-[1,4,2,5]dioxadiazine

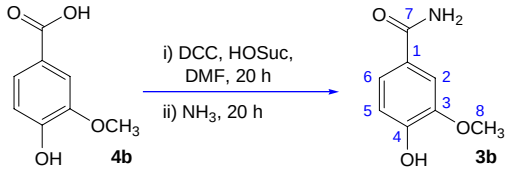
Preparation of 3,6-diphenyl-[1,4,2,5]dioxadiazine **11a**[38] Triethylamine (0.88 ml, 6.3 mmol) was added to a solution of benzaldehyde oximyl chloride **7a** (0.49 g, 3.15 mmol) and pyridine (0.51 ml, 6.3 mmol) dissolved in ethanol (10 ml). After 20 h the reaction mixture was added to dichloromethane (50 ml) and transferred to a separating funnel. The organic solution was washed with water (10 ml), copper sulphate solution (10 ml) and water (10 ml) and dried with anhydrous sodium sulphate. The residue from evaporation was purified by column chromatography, eluting with 1:1 petroleum ether: diethyl ether, to give 3,6-diphenyl-[1,4,2,5]dioxadiazine **11a** (0.15 g, 40 %) as a pale yellow powder. δ_H ($CDCl_3$) 7.42 (4H, t *J* 7.7, 4-H_4); 7.51 (2H, tt *J* 7.5, 1.2, 5-H_2); 7.87 (4H, dd, *J* 7.9, 1.0, 3-H_4); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 125.6 (C, 2-C); 127.3 (CH, 7.87, 3-C); 128.9 (CH, 7.42, 4-C); 132.9 (CH, 7.51, 5-C); 162.7 (C, 1-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 125.6 (7.42, 2-C); 127.3 (7.87, 7.51, 3-C); 128.9 (7.42, 4-C); 132.9 (7.87, 5-C); 162.7 (7.87, 1-C); ν_{max} (KBr) 1612[38], 1570, 1444, 1338[38], 1084[38], 1068, 1025, 845[38], 773, 691; mpt. 88-92 °C (lit. mpt. 101-102)[38,43]; *m/z* (EI+, TOF) 238 (3, M+; $C_{14}H_{10}N_2O_2$ requires 238.0742; found 238.0745, +1.1 ppm error); 222 (16, M – O; $C_{14}H_{10}N_2O$ requires 222.0793; found 222.0794, +0.5 ppm error); 119 (3, PhCON, C_7H_5NO requires 119.0371; found

119.0381, +8 ppm error); 103 (100, PhCN; C_7H_5N requires 103.0422; found 103.0405, -16 ppm error); 76 (38, C_6H_4).

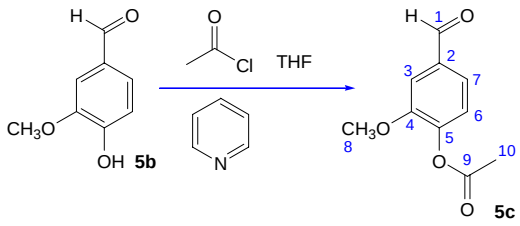


Preparation of 3,4-diphenyl-1,2,5-oxadiazol-2-oxide **9a** *and 5-H-3,5-diphenyl[1,2,4]oxadiazol-4-ol* **12a** A solution of benzaldehyde oximyl chloride **7a** (0.5 g, 3.2 mmol) in diethyl ether (10 ml) was shaken vigorously with saturated sodium carbonate solution (10 ml). The organic layer was dried with anhydrous magnesium sulphate and split into two equal portions. The first portion was monitored by ^{1}H NMR which showed the formation of 3,4-diphenyl-1,2,5-oxadiazol-2-oxide **9a** after 20 h. Work-up as reported previously gave pure product (0.11 g, 58 % yield). The second portion was added to a solution of (*Z*)-benzaldoxime **1a** (0.2 g, 1.65 mmol) in diethyl ether (5 ml). After 20 h the reaction mixture was evaporated and the crude residue purified by column chromatography, eluting with 7:3 petroleum ether: diethyl ether, to give 5-*H*-3,5-diphenyl[1,2,4]oxadiazol-4-ol **12a**. Recrystallisation from THF: hexane gave small white needle crystals (0.12 g, 32 %). Samples dissolved in $CDCl_3$ decomposed. *5-H-3,5-Diphenyl[1,2,4]oxadiazol-4-ol* ***12a*****:** δ_H (C_6D_6) 6.08 (1H, s, 1-H); 7.05 (3H, m, 9-H_2, 10-H); 7.10 (3H, m, 4-H_2, 5-H); 7.45 (2H, m, 3-H_2); 7.77 (2H, m, 8-H_2); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 101.7 (CH, 6.08, 1-C); 126.1 (C, 7-C); 127.5 (CH, 7.45, 3-C); 127.7 (CH, 7.77, 8-C); 128.5 (CH, 7.10, 4-C); 128.6 (CH, 7.05, 9-C); 129.4 (CH, 7.10, 5-C); 130.5 (CH, 7.05, 10-C); 135.9 (C, 2-C); 159.2 (C, 6-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 101.7 (7.45, 1-C); 126.1 (7.05, 7-C); 127.5 (7.10, 6.08, 3-C); 127.7 (7.05, 8-C); 128.5 (7.10, 4-C); 128.6 (7.05, 9-C); 129.4 (7.45, 5-C); 130.5 (7.77, 10-C); 135.9 (7.10, 2-C); 159.2 (7.77, 6.08, 6-C); ν_{max} (KBr) 3288; 1494; 1452; 1348; 1296; 744; 691; Mpt. 135 - 136 °C (lit. mpt. 141 – 143 °C)[45]; *m/z* (EI+, TOF) 222 (28, M – H_2O; $C_{14}H_{10}N_2O$ requires 222.0793; found 222.0795, +0.8 ppm error); 119 (37, C_7H_5NO[54]); 105 (38, C_7H_5O requires 105.0340; found 105.0347, +6 ppm error); 103 (100, PhCN; C_7H_5N requires 103.0422; found 103.0417, -5 ppm error); 77 (45, C_6H_5 requires 77.0391; found 77.0385, -8 ppm error).

Preparation of vanillin derivatives

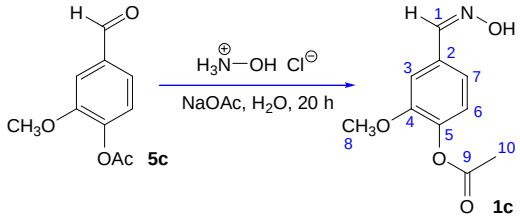


Vanillamide **3b** Dicyclohexylcarbodiimide (13.5g, 65.5 mmol), pyridine (10.1 cm^3, 125mmol) and *N*-hydroxysuccinimide (7.5g, 65.2 mmol) were added to a solution of vanillic acid **4b** (10 g, 59.5 mmol) in DMF (50 cm^3) cooled in an ice bath. The reaction was allowed to rise to room temperature and stirred for 20 h. The precipitate (DCU) was removed by filtration and washed with DMF (2 x 5 cm^3). The collected filtrate was added to concentrated ammonia solution (150 cm^3), which was vigorously stirred for 20 h. The resultant precipitate was collected by filtration and recrystallised from hot ethanol to give transparent needles (6.83 g, 69 % mpt 148 – 150 °C; lit. mpt 153 – 154 °C[37]). δ_H (DMSO-d_6) 3.8 (3H, s, 8-H_3); 6.84 (1H, d *J* 8.1, 5-H); 7.12 (1H, broad s, NH); 7.37 (1H, dd *J* 8.2, 2.0, 6-H); 7.45 (1H, d *J* 2.0, 2-H); 7.77 (1H, broad s, NH); 9.5 (1H, s, OH); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY) 55.5 (CH_3, 8-C); 111.5 (CH, 7.45, 2-C); 114.7 (CH, 6.84, 5-C); 121.1 (CH, 7.37, 6-C); 124.9 (C, 1-C); 147.1 (C, 3-C); 149.9 (C, 4-C); 167.7 (C, 7-C)[89]; *m/z* (EI+) 167.1 (M^+. 48); 151 (63); 123.1 (22); 108.1 (27); 96.2 (38) ; 53.2 (58); 52.3 (100); 51.2 (90); *m/z* (CI+, NH_3) 185.1 (M + NH_4, 62); 168.0 (M + H, 100; $C_8H_{10}O_3N$ requires 168.0655; found 168.065); ν_{max} (KBr/cm^{-1}) 3446.2 (str. sh., OH); 3321.4 (str. br., NH); 1652.7 (str. sh., amide-C=O); 1606.4 (str. sh., ar-C=C); 1581.7 (str. sh., ar-C=C); 1521.6 (str. sh., ar-C=C).

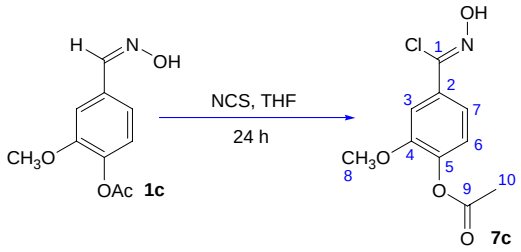


4-O-Acetyl vanillin **5c** A solution of acetyl chloride (13.8 ml, 15.24 g, 0.195 mol) in THF (50 ml) was added dropwise to a ice cooled solution of vanillin **5b** (20 g, 0.13 mol) in THF (100 ml). When addition was complete, a solution of pyridine (15.8 ml, 15.45 g, 0.196 mol) in THF (50 ml) was also added dropwise. The reaction was stirred for 20 h. The precipitate was removed by filtration and washed with diethyl ether (50 ml), the filtrate was reduced by rotary evaporation, and the residue dissolved in hot THF and filtration repeated. Finally the filtrate evaporated, dissolved in dichloromethane (150 ml) and washed with water (20 ml), saturated copper (II) sulphate solution (20 ml) and water (20 ml). The organic layer was then dried with anhydrous sodium sulphate and the solvent evaporated to give 4-*O*-acetyl vanillin (22.75 g, 90 %) as a white powder. The product showed no contaminants by NMR and was used without further purification. *4-O-Acetyl vanillin* **5c**: δ_H

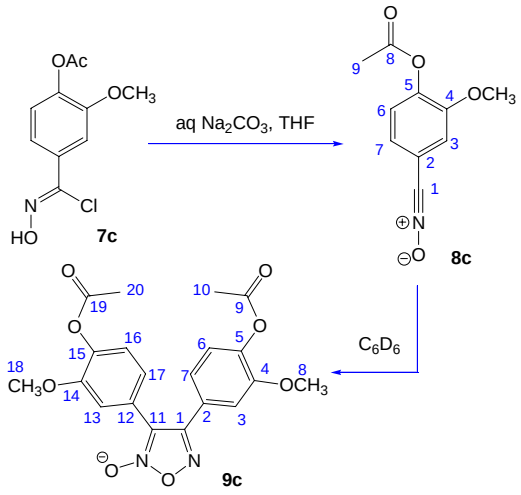
($CDCl_3$) 2.28 (3H, s, 10-H_3); 3.84 (3H, s, 8-H_3); 7.15 (1H, d *J* 7.9, 6-H); 7.41 (1H, dd *J* 7.9, 1.7, 7-H); 7.44 (1H, d *J* 1.7, 3-H); 9.88 (1H, s, 1-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 20.7 (CH_3, 2.28, 10-C); 56.1 (CH_3, 3.84, 8-C); 110.8 (CH, 7.44, 3-C); 123.4 (CH, 7.15, 6-C); 124.8 (CH, 7.41, 7-C); 135.2 (C, 2-C); 144.9 (C, 5-C); 152.0 (C, 4-C); 168.4 (C, 9-C); 191.1 (CH, 9.88, 1-C)[90]; δ_C (^{13}C -^{1}H- ^{3}*J*-COSY) 110.8 (9.88, 7.41, 3-C); 124.8 (7.44, 7-C); 135.2 (7.15, 2-C); 144.9 (7.44, 7.41, 5-C); 152.0 (7.15, 3.84, 4-C); 191.1 (7.44, 7.41, 1-C); ν_{max} (KBr) 1758; 1691; 1599; 1508; mpt 74 – 76 °C (lit. mpt. 77 °C)[91,92]; m/z (EI+, TOF) 194 (6, M^+; $C_{10}H_{10}O_4$ requires 194.0579; found 194.0582, +1.5 ppm error); 153 (7); 152 (100, M – ketene; $C_8H_8O_3$ requires 152.0473; found 152.0461, -8 ppm error); 151 (92, M – CH_3CO; $C_8H_7O_3$ requires 151.0395; found 151.0386, -6 ppm error).



4-O-Acetyl-vanillin aldoxime **1c** A solution of hydroxylamine hydrochloride (8.0 g, 0.115 mol) in water (35 ml) and a solution of sodium acetate (11.51 g, 0.140 mol) in water (60 ml) were added to a solution of 4-*O*-acetyl vanillin **5c** (22.36 g, 0.115 mol) dissolved in a mixture of water (100 ml) and acetonitrile (100 ml). After 20 h the reaction mixture was transferred to a separating funnel and extracted with dichloromethane (3 x 100 ml). The organic extracts were combined, dried with anhydrous sodium sulphate, and evaporated to give a viscous yellow liquid. This was triturated with tetrahydrofuran and then recrystallised from diethyl ether to give pale yellow conglomerate crystals (21.03 g, 88 %) *4-O-acetyl-vanillin aldoxime* **1c** δ_H ($CDCl_3$) 2.25 (3H, s, 10-H_3); 3.78 (3H, s, 8-H_3); 7.00 (2H, m, 6-H, 7-H); 7.19 (1H, d *J* 2.2, 3-H); 8.03 (1H, s, 1-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 20.7 (CH_3, 2.25, 10-C); 55.9 (CH_3, 3.78, 8-C); 109.5 (CH, 7.19, 3-C); 120.8 (CH, 7.00, 7-C); 123.1 (CH, 7.00, 6-C); 130.9 (C, 2-C); 141.3 (C, 5-C); 149.8 (CH, 8.03, 1-C); 151.4 (C, 4-C); 168.9 (C, 9-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY) 109.5 (8.03, 7.00, 3-C); 120.8 (8.03, 7.19, 7-C); 130.9 (7.00, 2-C); 141.3 (7.19, 7.00, 5-C); 149.8 (7.19, 7.00, 1-C); 151.4 (7.00, 3.78, 4-C); ν_{max} (neat) 3397 (br. str.); 2942; 1765 (v. str.); 1600 (str.); 1511 (v. str); 1465; 1416; 1370; 1344; 1286; 1211 (v. str); mpt. 79 - 82ºC[93]; *m/z* (EI+, TOF) 209 (M^+, $C_{10}H_{11}NO_4$ requires 209.0688; found 209.0693, +2.4 ppm error); 167 (M - ketene; $C_8H_9NO_3$ requires 167.0582; found 167.0580, -1.4 ppm error); 149 (80, M – CH_3CO_2H; $C_8H_7NO_2$ requires 149.0476; found 149.0470, -5 ppm error); 134 (100, M – CH_3CO_2H – CH_3; $C_7H_4NO_2$ requires 134.0242; found 134.0235, -5 ppm error); 106 (95).



4-O-Acetyl-vanillin oximyl chloride **7c**[94] A solution of *N*-chlorosuccinimide (1.41 g, 10.6 mmol) dissolved in tetrahydrofuran (25 ml) was added to a solution of 4-*O*-acetyl vanillin aldoxime **1c** (2 g, 9.6 mmol) in tetrahydrofuran (25 ml). After 24 h dichloromethane (150 ml) was added and the organic solution washed with 1M hydrochloric acid (3 x 30 ml) and water (3 x 30 ml). The organic layer was dried with anhydrous sodium sulphate and the solvent removed by rotary evaporation to give quantitative recovery of *4-O-acetyl vanillin oximyl chloride* **7c** as a colourless gum (2.33 g, 100%), which was stored below 0 °C. δ_H ($CDCl_3$) 2.26 (3H, s, 10-H_3); 3.81 (3H, s, 8-H_3); 7.00 (1H, d *J* 8.8, 6-H); 7.38 (2H, m, 3-H, 7-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 20.7 (CH_3, 2.26, 10-C); 56.0 (CH_3, 3.81, 8-C); 110.9 (CH, 7.38, 3-C); 120.1 (CH, 7.38, 7-C); 122.8 (CH, 7.00, 6-C); 131.4 (C, 2-C); 138.6 (C, 1-C); 141.7 (C, 5-C); 151.0 (C, 4-C); 168.8 (C, 9-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY) 110.9 (7.38, 3-C); 120.1 (7.38, 7-C); 122.8 (6-C); 131.4 (7.00, 2-C); 141.7 (7.38, 5-C); 151.0 (7.00, 3.81, 4-C); ν_{max} (neat) 3258 (br., OH), 2976, 2876, 1768 (str., C=O), 1601, 1509, 1464, 1410, 1359, 1285, 1195 (str.), 1164 (str.), 1122, 1035, 1009, 905, 846, 817, 787.



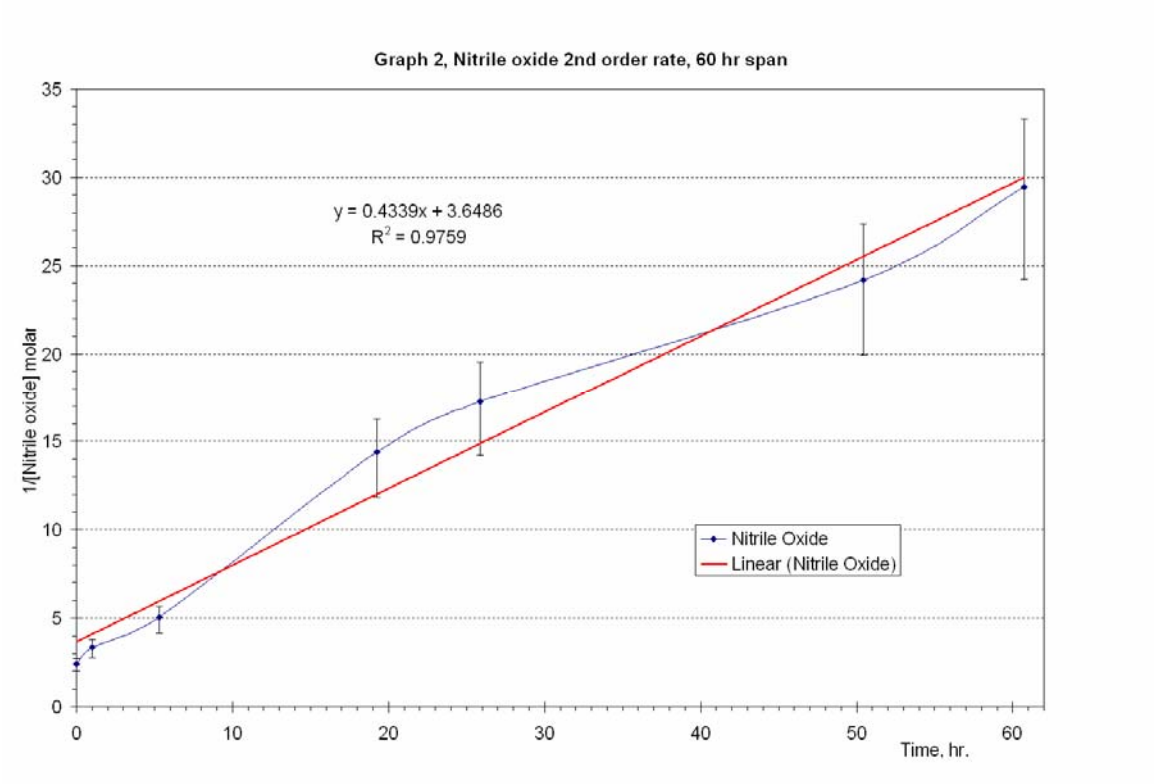
The preparation of 4-O-acetyl-vanillin nitrile oxide **8c** *and a kinetic study of the dimerisation to acetic acid 4-[4-(4-acetoxy-3-methoxy-phenyl)-2-oxy-furazan-3-yl]-2-methoxy-phenyl ester* **9c** A solution of 4-*O*-acetyl-vanillin oximyl chloride **7c** (0.1 g, 0.41 mmol) in tetrahydrofuran (2.5 ml) was added to a tin foil covered round bottomed flask containing saturated sodium carbonate solution (2.5 ml). The

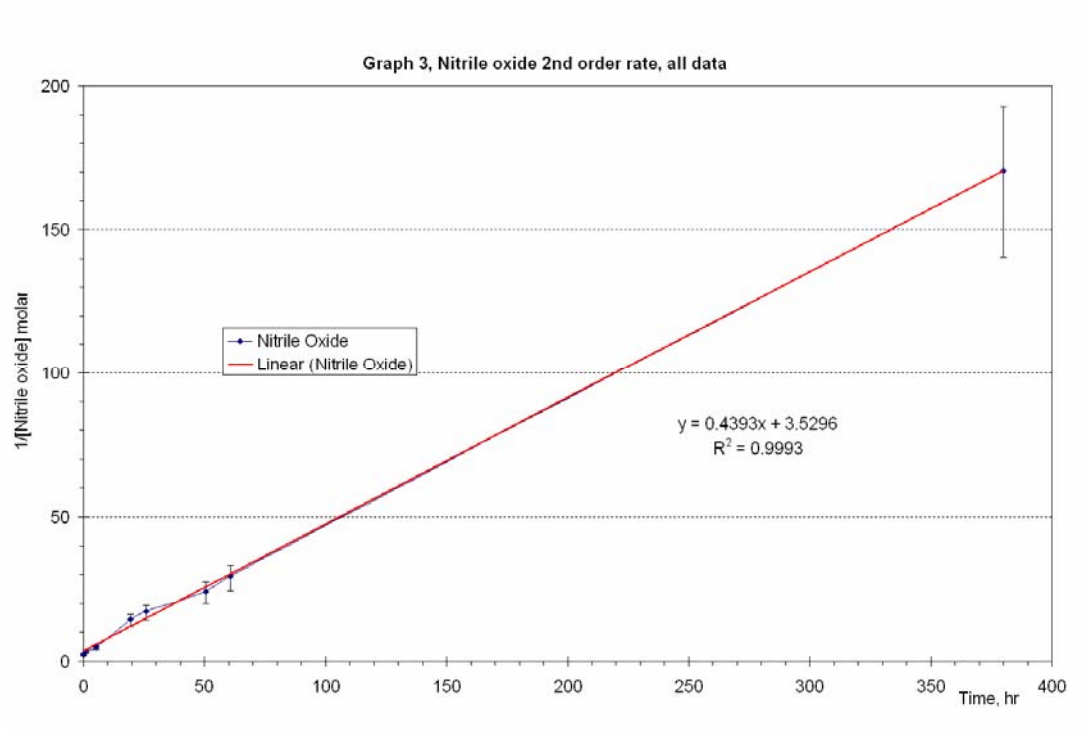
reaction was kept under nitrogen and vigorously stirred for 15 minutes. The reaction mixture was transferred to a separating funnel using dichloromethane (25 ml) and the organic layer removed. The organic layer was washed with water (5 ml), dried with anhydrous sodium sulphate and evaporated. The residue was immediately dissolved in deuterated benzene (0.75 ml) and analysed by ^{1}H-NMR, ^{13}C-NMR over 4 h, ^{1}H-NMR and HSQC and HMBC (Table 3). Conversion of nitrile oxide **8c** into the dimer **9c**, was approx. 80 % complete after 48 h. After circa 15 days the reaction mixture was evaporated to give a white solid (0.067 g, 79 %). The weight of product was used to calculate the initial weight of nitrile oxide **8c**, assuming no losses during workup.

Table 3, Kinetic data for the dimerisation of nitrile oxide **8c** to dimer **9c**

Time Decimal hr	% Nitrile oxide **8c**	% Dimer **9c**	molar Nitrile oxide **8c**	molar Dimer **9c**
0, estimated	100	0	0.41295	0.00000
1	83.7	16.3	0.29720	0.05788
5.33	64.9	35.1	0.19838	0.10729
19.27	28.8	71.2	0.06947	0.17174
25.88	24.6	75.4	0.05792	0.17752
50.45	18.2	81.8	0.04134	0.18581
60.75	15.2	84.8	0.03397	0.18949
380.07	2.8	97.2	0.00586	0.20354

The data was plotted as 1/[nitrile oxide] *vs* time and the regression line was $y = 0.434x + 3.65$ $R^2 = 0.98$. The error bars are set at ± 15 % of the relative proportions, calculated from the ^{1}H-NMR data.



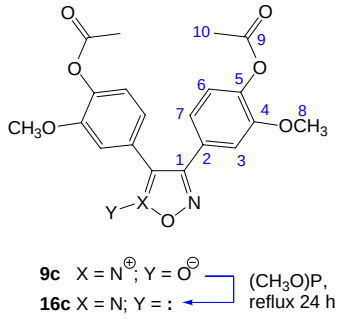


The results are largely unchanged when the last measurement is included.

4-O-acetyl-vanillin nitrile oxide **8c:** δ_H (C_6D_6) 1.85 (3H, s, 10-H_3); 3.10 (3H, s, 8-H_3); 6.30 (1H, d *J* 1.8, 3-H); 6.42 (1H, dd *J* 8.2, 1.8, 7-H); 6.63 (1H, d *J* 8.2, 6-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 20.2 (CH_3, 1.85, 10-C); 35.8 (C, t *J* circa 50, 1-C); 55.6 (CH_3, 3.10, 8-C); 112.2 (C, 2-C); 115.8 (CH, 6.30,

3-C): 123.9 (CH, 6.63, 6-C); 125.0 (CH, 6.42, 7-C); 142.5 (C, 5-C); 152.0 (C, 4-C); 167.6 (C, 9-C); δ_C (^{13}C -^{1}H- ^{3}J-COSY); 20.2 (10-C); 35.8 (6.42, 6.30, 1-C); 55.6 (8-C); 112.2 (6.63, 2-C); 115.8 (6.42, 3-C): 123.9 (6-C); 125.0 (6.30, 7-C); 142.5 (6.42, 6.30, 5-C); 152.0 (6.63, 4-C); 167.6 (9-C);

Acetic acid 4-[4-(4-acetoxy-3-methoxy-phenyl)-2-oxy-furazan-3-yl]-2-methoxy-phenyl ester **9c** δ_H (C_6D_6) 1.86 (3H, s, 10-H_3 or 20-H_3); 1.87 (3H, s, 10-H_3 or 20-H_3); 3.24 (3H, s, 8-H_3 or 18-H_3); 3.26 (3H, s, 8-H_3 or 18-H_3); 6.81 (3H, m, 6-H, 13-H, 16-H); 6.84 (1H, d *J* 1.8, 3-H); 6.92 (1H, dd *J* 8.1, 1.8, 7-H); 7.04 (1H, dd *J* 8.2, 1.9, 17-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}J-COSY); 20.1 (2CH_3, 1.86, 1.87, 10-C, 20-C); 55.6 (2CH_3, 3.24, 3.26, 8-C, 18-C); 112.8 (CH, 6.81, 13-C); 112.9 (CH, 6.84, 3-C); 113.6 (C, 11-C); 121.3 (CH, 6.92, 7-C); 121.5 (CH, 7.04, 17-C); 121.6 (C, 2-C); 123.7 (CH, 6.81, 6-C or 16-C); 123.9 (CH, 6.81, 6-C or 16-C); 125.6 (C, 12-C); 142.0 (C, 15-C); 142.5 (C, 5-C); 151.9 (C, 4-C or 14-C); 152.1 (C, 4-C or 14-C); 155.8 (C, 1-C); 167.8 (2C, 9-C, 19-C); δ_C (^{13}C -^{1}H- ^{3}J-COSY); 20.1 (10-C + 20-C); 55.6 (8-C + 18-C); 112.8 (7.04, 13-C); 112.9 (6.92, 3-C); 113.6 (7.04, 6.81, 11-C); 121.3 (6.84, 7-C); 121.5 (6.81, 17-C); 121.6 (6.81, also ^{2}J with 3-H, 6.84, 2-C); 123.7 (6-C or 16-C); 123.9 (6-C or 16-C); 125.6 (6.81, 12-C); 142.0 (7.04, 6.81, 15-C); 142.5 (6.92, 6.84, 5-C); 151.9 (6.81, 4-C or 14-C); 152.1 (6.81, 4-C or 14-C); 155.8 (6.92, 6.84, 1-C); 167.8 (9-C, 19-C). Mpt. 141-144 °C. ν_{max} (KBr) 2964, 2934, 1759, 1610, 1593, 1516, 1451, 1437, 1373, 1259, 1219, 1197, 1174, 1156, 1114, 1031, 867, 784.

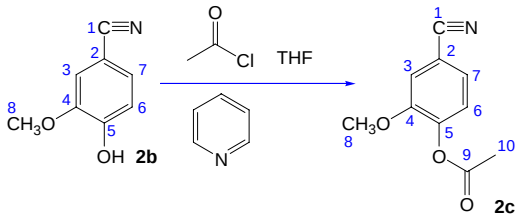


9c X = $N^{\oplus}$; Y = $O^{\ominus}$ → $(CH_3O)P$, reflux 24 h
16c X = N; Y = **:** ←

Trimethyl phosphite deoxygenation of acetic acid 4-[4-(4-acetoxy-3-methoxy-phenyl)-2-oxy-furazan-3-yl]-2-methoxy-phenyl ester ***9c*** *to acetic acid 4-[4-(4-acetoxy-3-methoxy-phenyl)-furazan-3-yl]-2-methoxy-phenyl ester* ***16c*** The dimer **9c** (54 mg, 0.13 mmol) was refluxed for 24 h in neat trimethyl phosphite (2 ml, 16.9 mmol). The reaction mixture was placed under rotary evaporation; dry toluene was added to remove the last traces of trimethyl phosphite and the evaporation repeated. The residue was triturated with diethyl ether and filtered to give the symmetrical species; acetic acid 4-[4-(4-acetoxy-3-methoxy-phenyl)-furazan-3-yl]-2-methoxy-phenyl ester **16c** as a white solid (31 mg, 60 %).

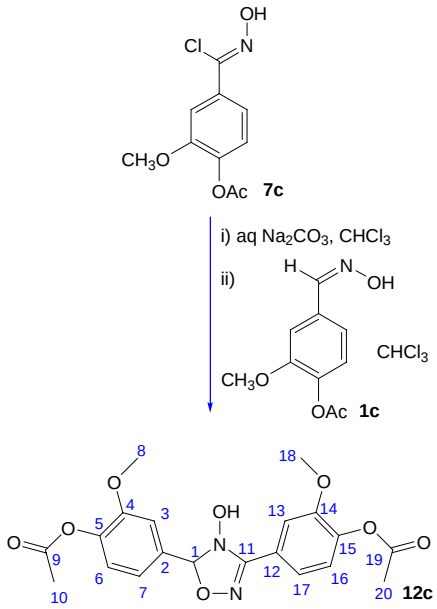
Acetic acid 4-[4-(4-acetoxy-3-methoxy-phenyl)-furazan-3-yl]-2-methoxy-phenyl ester **16c:** δ_H (C_6D_6) 1.82 (6H, s, 10-H_3); 3.16 (6H, s, 8-H_3); 6.77 (2H, d *J* 8.2, 6-H); 6.90 (2H, d *J* 1.9, 3-H); 6.98 (2H, dd, *J*

8.2, 1.9, 7-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 20.0 (CH_3, 1.82, 10-C); 55.4 (CH_3, 3.16, 8-C); 113.2 (CH, 6.90, 3-C); 121.6 (CH, 6.98, 7-C): 123.8 (CH, 6.77, 6-C); 124.5 (C, 2-C); 142.2 (C, 5-C); 152.0 (C, 4-C); 152.9 (C, 1-C); 167.6 (C, 9-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 20.0 (10-C); 55.4 (8-C); 113.2 (6.98, 3-C); 121.6 (6.90, 7-C): 123.8 (6-C); 124.5 (6.77, 2-C); 142.2 (6.98, 6.90, 5-C); 152.0 (6.77, 3.16, 4-C); 152.9 (6.98, 6.90, 1-C); 167.6 (9-C); ν_{max} (KBr) 2972, 1766, 1588, 1518, 1479, 1373, 1264, 1242, 1202, 1173, 1124, 1099, 1011, 995, 860, 801, 749, 690; *m/z* (ESI+) 416 (13, M + NH_4^+, $C_{20}H_{22}O_7N_3$ requires 416.1452, found 416.1456, +0.9 ppm error); 302 (11); 300 (10); 233 (42); 141 (100); (APCI+) 438 (18); 437 (85, M + K, $C_{20}H_{18}N_2O_7K$ requires 437.0751, found 437.0736, -3.5 ppm error); 421 (98, M + Na); 416 (100, M + NH_4); 399 (M + H, 75); 357 (86); Mpt. 128-130 °C.



The preparation of 4-cyano-2-methoxyphenyl acetate (4-O-acetyl-vanillin nitrile) **2c** Vanillin nitrile **2b** (2 g, 13.4 mmol) was dissolved in tetrahydrofuran (20 ml). The solution was cooled in an ice bath then acetyl chloride (1.43 ml, 20.1 mmol) and pyridine (1.63 ml, 20.1 mmol) were added dropwise by syringe. Once the addition was complete the solution was allowed to warm to room temperature. After 2 h the reaction mixture was transferred to a separating funnel, combined with dichloromethane (50 ml), and washed with copper sulphate solution (2 x 20 ml) and water (2 x 20 ml). The organic layer was subsequently dried with anhydrous sodium sulphate and the solvent evaporated to yield 4-*O*-acetyl vanillin nitrile **2c** as a white solid (2.32 g, 91 %). NMR analysis indicated the material was pure.
Vanillin nitrile (Aldrich) ***2b:*** δ_H (C_6D_6) 2.86 (3H, s, 8-H_3); 5.80 (1H, br. s, 5-OH); 6.44 (1H, d *J* 1.7, 3-H); 6.55 (1H, d *J* 8.2, 6-H); 6.70 (1H, dd *J* 8.2, 1.7, 7-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 55.3 (CH_3, 2.86, 8-C); 103.7 (C, 2-C); 113.8 (CH, 6.44, 3-C); 115.2 (CH, 6.55, 6-C); 119.2 (C, 1-C); 126.8 (CH, 6.70, 7-C); 146.7 (C, 4-C); 150.0 (C, 5-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 55.3 (8-C); 103.7 (6.55, 2-C); 113.8 (6.70, 3-C); 115.2 (6-C); 119.2 (6.70, 6.44, 1-C); 126.8 (6.44, 7-C); 146.7 (6.55, 4-C); 150.0 (6.70, 6.44, 2.86, 5-C).[95]
4-O-Acetyl vanillin nitrile ***2c*****:** δ_H (C_6D_6) 1.80 (3H, s, 10-H_3); 2.96 (3H, s, 8-H_3); 6.56 (1H, d *J* 1.6, 3-H); 6.59 (1H, d *J* 8.1, 6-H); 6.68 (1H, dd *J* 8.1, 1.7, 7-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 19.7 (CH_3, 1.80, 10-C); 55.1 (CH_3, 2.96, 8-C); 110.6 (C, 2-C); 115.3 (CH, 6.56, 3-C); 118.1 (C, 1-C); 123.6 (CH, 6.59, 6-C); 124.9 (CH, 6.68, 7-C); 143.6 (C, 5-C); 151.7 (C, 4-C); 167.0 (C, 9-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 19.7 (10-C); 55.1 (8-C); 110.6 (6.59, 2-C); 115.3 (6.68, 3-C); 118.1 (6.68, 6.56, 1-C); 123.6

(6-C); 124.9 (6.56, 7-C); 143.6 (6.68, 6.56, 5-C); 151.7 (6.59, 2.96, 4-C); 167.0 (9-C); ν_{max} (KBr) 1756, 1602, 1510, 1472, 1411, 1381, 1290, 1208, 1152, 1125, 1029, 941, 905, 862, 830, 792; Mpt 110 - 111 °C.



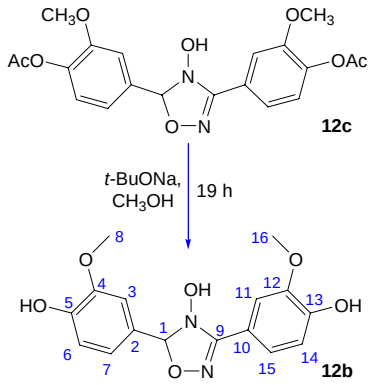
*Attempted preparation of cycloadduct **12 c** with boron trifluoride etherate catalysis* 4-*O*-Acetyl-vanillin oximyl chloride **7c** (0.25 g, 1.03 mmol) was stirred with saturated sodium carbonate in diethyl ether (2.5 mls). The reaction mixture was added to dichloromethane (30 ml), washed with water (2 x 10 ml) and evaporated to give an oily material (0.32 g). A small portion was dissolved in C_6D_6 and analysed by ^{1}H-NMR which indicated a 75:25 ratio of nitrile oxide **8c**: dimer **9c**. The remainder was added to a solution of 4-*O*-acetyl vanillin aldoxime **1c** (0.215 g, 1.03 mmol) and $BF_3.Et_2O$ (0.13 mls, 0.146 g, 1.03 mmol) in ether (5 ml). After 2 hours a ^{1}H-NMR spectrum (C_6D_6) of an aliquot, showed a 50:50 mixture of nitrile oxide **8c**: 4-*O*-acetyl vanillin aldoxime **1c** and after 60 hours a 23:73 ratio of dimer **9c**: 4-*O*-acetyl vanillin aldoxime **1c**. No traces of nitrile oxide **8c** or the cyclo-adduct **12c** were detected.

Stability of 4-O-acetyl vanillin aldoxime **1c** *in boron trifluoride etherate* A mixture of $BF_3.Et_2O$ (0.061 ml, 0.068 g, 0.48 mmol) and 4-*O*-acetyl vanillin aldoxime **1c** (100 mg, 0.48 mmol) were stirred in ether (5 ml) for 2 days. The reaction mixture was added to dichloromethane (25 ml), washed with water (2 x 10 ml) and evaporated to give 4-*O*-acetyl vanillin aldoxime **1c** (100 mg, 100 %).

*Attempted preparation of cycloadduct **12 c** with excess 4-O-acetyl-vanillin aldoxime* **1c**

i) 4-*O*-Acetyl-vanillin oximyl chloride **7c** (0.5 g, 2.05 mmol) was dissolved in THF (5 ml) and added to saturated sodium carbonate solution (5 ml). The reaction mixture was added to dichloromethane (30 ml), washed with water (2 x 10 ml) and evaporated to give an oil. A small aliquot was analysed by ^{1}H-NMR (C_6D_6, 85:15 ratio of nitrile oxide **8c**: dimer **9c**) and the remainder was added to 4-*O*-acetyl vanillin aldoxime **1c** (0.64 g, 3.075 mmol, 1.5 eq) in dichloromethane (5 ml). The reaction was monitored by evaporating aliquots and taking ^{1}H-NMR spectra (C_6D_6) which showed only nitrile oxide **9c** and aldoxime **1c**; 2.5 hrs, 21:79; 23.5 hrs 13:87; after work up 7:93.
ii) 4-*O*-Acetyl-vanillin oximyl chloride **7c** (0.25 g, 1.03 mmol) was dissolved in THF (2.5 ml) and added to saturated sodium carbonate solution (2.5 ml). The reaction mixture was added to dichloromethane (30 ml), washed with water (2 x 10 ml) and evaporated to give an oil (0.2 g), which was dissolved in C_6D_6 (1.5 ml, an aliquot analysed by ^{1}H-NMR, showed a 53: 47 ratio of nitrile oxide **8c**: dimer **9c**) and added to 4-*O*-acetyl vanillin aldoxime **1c** (0.323 g, 1.55 mmol, 1.5 eq) in C_6D_6 (0.75 ml). ^{1}H-NMR monitoring showed: 2 hrs 9:13:78 ratio of nitrile oxide **8c**: dimer **9c**: aldoxime **1c**; 17 hrs 18:82 ratio of dimer **9c**: aldoxime **1c**.

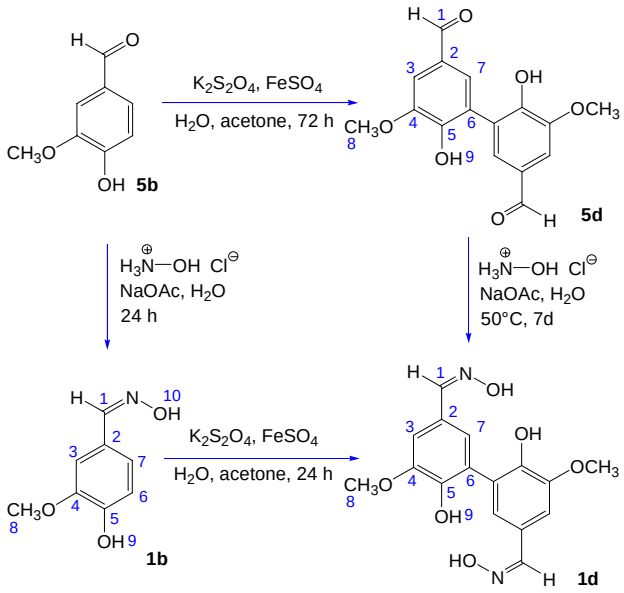
3,5-Di(4-acetoxy-3-methoxyphenyl)-5-H-[1,2,4]oxadiazol-4-ol **12c** 4-*O*-Acetyl-vanillin oximyl chloride **7c** (1.06 g, 4.4 mmol) was dissolved in chloroform (10 ml) and treated with saturated sodium carbonate solution (10 ml). After 15 mins the reaction mixture was transferred to a separating funnel. The organic layer was removed and the aqueous layer was extracted with chloroform (2 x 10 ml). The organic layers were combined, dried with anhydrous sodium sulphate, and decanted into a solution of 4-*O*-acetyl vanillin aldoxime **1c** (1.38 g, 6.6 mmol) in chloroform (10 ml). After 18 h the solvent was removed by rotary evaporation and the crude residue purified by column chromatography, eluting with 7:3 diethyl ether: petroleum ether to give *3,5-di(4-acetoxy-3-methoxyphenyl)-5-H-[1,2,4]oxadiazol-4-ol* **12c** (0.18 g, 10 %) as a white powder. δ_H ($CDCl_3$) 2.23 (6H, m, 10-H_3, 20-H_3); 3.72 (6H, m, 8-H_3, 18-H_3); 6.07 (1H, s, 1-H); 6.96 (1H, d *J* 8.1, 6-H); 6.98 (1H, d *J* 8.2, 16-H); 7.04 (1H, *J* dd 8.1, 1.7, 7-H); 7.07 (1H, d *J* 1.7, 3-H); 7.34 (1H, dd *J* 8.2, 1.7, 17-H); 7.39 (1H, d *J* 1.7, 13-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 20.7 ($2CH_3$, 2.23, 10-C, 20-C); 56.1 ($2CH_3$, 3.72, 8-C, 18-C); 101.1 (CH, 6.07, 1-C); 111.1 (CH, 7.07, 3-C); 111.5 (CH, 7.39, 13-C); 119.8 (CH, 7.04, 7-C); 120.5 (CH, 7.34, 17-C); 122.8 (CH, 6.96, 6-C); 123.2 (CH, 6.98, 16-C); 123.9 (C, 12-C); 134.3 (C, 2-C); 140.6 (C, 5-C); 141.9 (C, 15-C); 151.2 (2C, 4-C, 14-C); 159.2 (C, 11-C); 169.0 (C, 9-C or 19-C); 169.4 (C, 9-C or 19-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY) 101.1 (7.07, 7.04, 1-C); 111.1 (7.04, 6.07, 3-C); 111.5 (7.34, 13-C); 119.8 (7.07, 6.07, 7-C); 120.5 (7.39, 17-C); 123.9 (6.98, 12-C); 134.3 (6.96, 2-C); 140.6 (7.07, 7.04, 5-C); 141.9 (7.39, 7.34, 15-C); 151.2 (6.96, 3.72, 4-C, 6.98, 3.72, 14-C); 159.2 (7.39, 7.34, 6.07, 11-C); 169.0 (9-C or 19-C); 169.4 (9-C or 19-C).



3,5-Di(4-hydroxy-3-methoxyphenyl)-5-H-[1,2,4]oxadiazol-4-ol **12b** Sodium *t*-butoxide (25 mg, 0.26 mmol) was added to a solution of 3,5-di(4-acetoxy-3-methoxyphenyl)-5-*H*-[1,2,4]oxadiazol-4-ol **12c** (0.18 g, 0.43 mmol) dissolved in methanol (10 ml). The pH of the solution was maintained at >= 10 (as judged from universal indicator paper) by addition of further portions of sodium *t*-butoxide. After 19 hours dichloromethane (40 ml) was added and the solution washed with 1M hydrochloric acid solution (15 ml) and water (2 x 15 ml). The organic layer was dried with anhydrous sodium sulphate and the solvent removed by rotary evaporation. The crude product was purified by column chromatography, eluting with 7:3 diethyl ether: petroleum ether, to give *3,5-di(4-hydroxy-3-methoxyphenyl)-5-H-[1,2,4]oxadiazol-4-ol* **12b** (0.04 g, 28 %) as a pale yellow powder. δ_H (C_6D_6) 3.13 (3H, s, 8-H_3 or 16-H_3); 3.18 (3H, s, 8-H_3 or 16-H_3); 5.66 (1H, s, OH, 5-C); 5.73 (1H, s, OH, 13-C); 6.23 (1H, s, 1-H); 7.08 (1H, m, 7-H); 7.10 (2H, m, 6-H, 14-H); 7.18 (1H, d *J* 1.7, 3-H); 7.38 (1H, dd *J* 8.3, 1.8, 15-H); 7.55 (1H, d *J* 1.8, 11-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 55.0 (2CH_3, 3.13, 3.18, 8-C, 16-C); 101.7 (CH, 6.23, 1-C); 109.8 (CH, 7.55, 11-C); 109.9 (CH, 7.18, 3-C); 114.2 (CH, 7.10, 6-C); 114.4 (CH, 7.10, 14-C); 117.9 (C, 10-C) 121.4 (CH, 7.08, 7-C); 121.8 (CH, 7.38, 15-C); circa 128 (obscured by C_6D_5H, 2-C); 146.8 (2C, 4-C, 12-C); 147.3 (C, 5-C); 148.6 (C, 13-C); 159.6 (C, 9-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 101.7 (7.18, 7.08, 1-C); 109.8 (7.38, 11-C); 109.9 (7.08, 6.23, 3-C); 114.2 (5.66, 6-C); 114.4 (5.73, 14-C); 117.9 (7.10, 2-C, 10-C); 121.4 (7.18, 6.23, 7-C); 121.8 (7.55, 15-C); ~128 (7.10, 2C); 146.8 (3.13, 3.18, 4-C, 12-C); 147.3 (7.18, 7.08, 5-C); 148.6 (7.55, 7.38, 13-C); 159.6 (6.23, 7.55, 7.38, 9-C); ν_{max} (KBr) 3411 (br. str.); 2926; 1603; 1516 (v. str); 1465; 1430; 13 44; 1267; Mpt. 46 – 48 °C; additional NMR data δ_H (DMSO-d_6) 3.79 (3H, s, 8-H_3); 3.81 (3H, s, 16-H_3); 5.95 (1H, s, 1-H); 6.83 (1H, d *J* 8.2, 6-H); 6.87 (1H, d *J* 8.2, 14-H); 6.98 (1H, dd *J* 8.2, 1.8, 7-H); 7.10 (1H, d *J* 1.8, 3-H); 7.28 (1H, dd *J* 8.2, 1.8, 15-H); 7.31 (1H, *J* 1.8, 11-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 56.2 (CH_3, 3.79, 3.81, 8-C, 16-C); 101.1 (CH, 5.95, 1-C); 111.3 (CH, 7.31, 11-C); 111.9 (CH, 7.10, 3-C); 115.7 (CH, 6.83, 6-C); 115.9 (CH, 6.87, 14-C); 116.6 (C, 10-C); 120.9 (CH, 6.98, 7-C); 121.2 (CH, 7.28, 15-C); 127.0 (C, 2-C); 148.0 (2C, 4-C, 12-C); 148.1 (C, 5-C); 149.6 (C, 13-C); 160.2 (C, 9-C); δ_C (^{13}C -

^{1}H- ^{3}*J*-COSY); 56.2 (8-C, 16-C); 101.1 (7.10, 6.98, 1-C); 111.3 (7.28, 11-C); 111.9 (6.98, 3-C); 115.7 (6-C); 115.9 (14-C); 116.6 (6.87, 10-C); 120.9 (7.10, 7-C); 121.2 (7.31, 15-C); 127.0 (6.83, 2-C); 148.0 (6.87, 6.83, 3.81, 3.79, 4-C, 12-C); 148.1 (7.10, 6.98, 5-C); 149.6 (7.31, 7.28, 13-C); 160.2 (7.31, 7.28, 5.95, 9-C);

Phenolic oxidative coupling products



6,6´-Dihydroxy-5,5´-dimethoxy-biphenyl-3,3´-dicarbaldehyde **5d** *by phenolic oxidative coupling of vanillin* **5b.** Vanillin **5b** (1 g, 6.6 mol) was dissolved in a solution of water (20 ml) and acetone (1 ml). Once fully dissolved potassium persulphate (1.09 g, 4.05 mmol) and iron sulphate (0.049 g, 0.26 mmol) were added in one portion. After 72 h the resulting precipitate was removed by filtration, washed with water (2 x 10 ml) and diethyl ether (2 x 10 ml), to give crude dehydro-dimer (0.76 g). This solid contained unreacted vanillin **5b** and was purified by washing with hot methanol, yielding 6,6´-dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dicarbaldehyde (0.47, 47 %) and the methanol washings contained vanillin (0.23 g, 23 %).

Vanillin **5b** (Aldrich): 0.04 mg ml^{-1}, CH_3OH, λ_{Max} nm (ε); 209 (11,790); 230 (12,520); 278.5 (10,760); 307.5 (10,410); HPLC detector λ 254 (2,967) detection at this wavelength is not recommended, because the absorption has a minimum at 248 nm. This data is similar to that reported for vanillin **5b** in 0.01 N HCl.[96]

6,6´-Dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dicarbaldehyde **5d:** δ_H (DMSO-d_6) 3.94 (6H, s, 8-H_6); 7.44 (4H, s, 3-H_2, 7-H_2); 9.82 (2H, s, 1-H_2); 9.88 (2H, br. s, 9-H_2); δ_C (DEPT, δ_H ^{1}H-^{13}C); 56.5 (CH_3, 3.94, 8-C); 109.5 (CH, 7.45, 3-C, csp); 125.0 (C, C-2 or C-6); 128.1 (CH, 7.45, 7-C, csp); 128.6 (C, C-

2 or C-6); 148.6 (C, 4-C); 151.0 (C, 5-C); 191.6 (CH, 9.85, 1-C); ν_{max} (KBr) 3249; 1672; 1587; 1503; 1454; 1422; 1354; 1311; 1258; 1148; 1044; 919; 882; 846; 771; 743; 655; 0.038 mg ml^{-1}, CH_3OH, λ_{Max} nm (ε), 231.5 (13,650); 287 (11,610); 307.5 (11,720); HPLC detector λ 254 (11,290); mpt. 291 – 292 °C. (lit. mpt. 300 - 305 °C). This material was identical to that purchased from Aldrich as judged by TLC, ^{1}H- and ^{13}C-DEPT NMR.

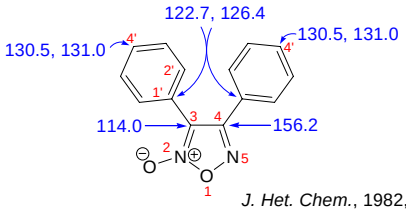
6,6´-Dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dialdoxime **1d** *from 6,6´-dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dicarbaldehyde* **5d** *and hydroxylamine* A solution of hydroxylamine hydrochloride (58 mg, 0.83 mmol) in water (2 ml) and a solution of sodium acetate (82 mg, 1.0 mmol) in water (2 ml) were added to a solution of 6,6´-dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dicarbaldehyde **1d** (0.25 g, 0.83 mmol) dissolved in water (20 ml) and acetonitrile (5 ml). The reaction mixture was kept at 50 °C for 7 days and monitored by ^{1}H NMR; 40 % conversion to the aldoxime product was observed. DMF (5 ml) was added to increase the solubility of the components, the solution was cooled, the excess liquid decanted away from the precipitate, and the remaining solvent removed by rotary evaporation to give 6,6´-dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dialdoxime **1d** as a white powder (0.09 g, 33 %). The ^{1}H- and ^{13}C-NMR data were identical to that of material produced by phenolic oxidative coupling of vanillin aldoxime.

Vanillin aldoxime **1b** A solution of hydroxylamine hydrochloride (69.6 g, 1 mol) in water (200 ml) and a solution of sodium acetate (98.5 g, 1.2 mol) in water (200 ml) were added to a solution of vanillin **5b** (152 g, 1 mol) dissolved in water (200 ml). The reaction was stirred at room temperature for 24 h. The resulting precipitate was removed by filtration, washed with water (3 x 100 ml) and recrystallised from hot ethanol yielding *vanillin aldoxime* **1b** as white rhombic crystals (140.1 g, 83 %). δ_H ($CDCl_3$) 3.86 (3H, s, 8-H_3); 5.76 (1H, s, 10-H); 6.85 (1H, d *J* 8.1, 6-H); 6.92 (1H, dd *J* 8.1, 1.8, 7-H); 7.14 (1H, d *J* 1.8, 3-H); 7.99 (1H, s, 1-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 56.4 (CH_3, 3.86, 8-C); 107.9 (CH, 7.14, 3-C); 114.8 (CH, 6.92, 7-C); 122.8 (CH, 6.85, 6-C); 124.7 (C, 2-C); 147.3 (C, 4-C); 148.0 (C, 5-C); 150.8 (CH, 7.99, 1-C); ν_{max} (KBr) 3470; 3200 (v. br); 1608; 1518 (str. sh.); 1425; 1318; 1273 (str.); 1207; 1119; 1023; 978; 952; 821; 756; *m/z* (Q-TOF, ESI+, abundance) 168.1 (M + H, 30); 150.1 (100, MH - H_2O); 136.1 (20); 122.1 (18); 105.1 (8); 0.04 mg ml^{-1}, CH_3OH, λ_{Max} nm (ε), 218 (14,870); 270 (12,920); 300 (7,835); HPLC detector λ 254 (7,940); mpt. 117 – 118 °C, (lit. mpt. 118.2°C).[95,97,98,]

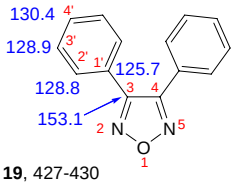
6,6´-Dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dialdoxime (vanillin aldoxime dehydro-dimer) **1d** *by phenolic oxidative coupling of vanillin aldoxime* **1b** Vanillin aldoxime **1b** (1 g, 6.0 mmol) was dissolved in a solution of water (20 ml) and acetone (5 ml). Once fully dissolved, potassium persulfate

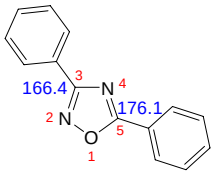
(0.99 g, 3.7 mmol) and iron sulphate (0.043 g, 0.23 mmol) were added in one portion. After 24 hrs the resulting precipitate was removed by filtration, washed with water (3 x 10 ml) and diethyl ether (3 x 10 ml), to give crude vanillin aldoxime dehydro-dimer **1d** (0.71 g, 71 %, circa 95 % purity). The crude solid (0.55 g) contained unreacted vanillin aldoxime was purified by washing with hot methanol to yield pure vanillin aldoxime dehydro-dimer **1d** (0.19 g, 19 %), plus impure material (0.34 g, 90 : 10; vanillin aldoxime dimer **1d**: vanillin aldoxime **1b**). *6,6´-Dihydroxy-5,5´-dimethoxybiphenyl-3,3´-dialdoxime* **1d**: δ_H (DMSO-d_6) 3.85 (3H, s, 8-H_3); 6.93 (1H, s, 7-H); 7.18 (1H, s, 3-H); 8.01 (1H, s, 1-H); 8.81 (1H, s, OH, 9-H); 10.86 (1H, s, OH, 10-H); δ_C (DEPT, δ_H ^{1}H-^{13}C ^{1}*J*-COSY); 56.2 (CH_3, 3.85, 8-C); 107.7 (CH, 7.18, 3-C); 123.3 (CH, 6.93, 7-C); 123.9 (C, 2-C); 125.6 (C, 6-C); 145.7 (C, 5-C); 148.3 (C, 4-C); 148.5 (CH, 8.01, 1-C); δ_C (^{13}C -^{1}H- ^{3}*J*-COSY); 56.2 (8-C); 107.7 (8.01, 6.93, 3-C); 123.3 (7.18, 7-C); 123.9 (2-C); 125.6 (8.81, 6.93, 6-C); 145.7 (7.18, 6.93, 5-C); 148.3 (3.85, 4-C); 148.5 (10.86, 7.18, 6.93, 1-C). ν_{max} (KBr) 3506, 1677, 1595, 1499, 1472, 1416, 1326, 1279, 1240, 1186, 1147, 1077, 1049, 984, 948, 850, 776, 688; 0.015 mg ml^{-1}, CH_3OH, λ_{Max} nm (ε); 233 (16,080); 269 (26,820); 294 (19,880); HPLC detector λ 254 (20,600); 0.03 mg ml^{-1}, CH_3OH, λ_{Max} nm (ε); 225.5 (24,330); 258.5 (23,790); 305 weak shoulder (11,405), very broad maxima almost indiscernible; mpt. 222 – 223 °C.

Appendix, NMR reference data



J. Het. Chem., 1982, **19**, 427-430





Het. Comm., 2004, **10**, 151-156.

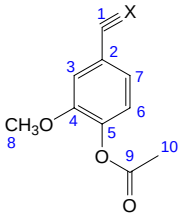
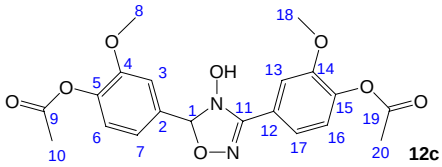


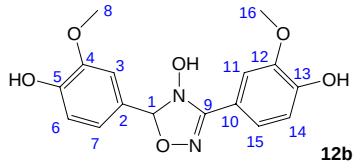
Table 4. Summary of ^{13}C-NMR shift data for vanillin derivatives

Carbon	1	2	3	4	5	6	7	8	9	10
Vanillin[90] **5b**	191.3	129.5	109.4	147.5	152.3	114.8	127.4	56.0	168.5	20.52
Vanillin* **5b**	191.4	129.2	111.1	148.6	153.5	115.9	126.5	56.0	-	-
Van. aldoxime	150.8	124.7	107.9	147.3	148.0	122.8	114.8	56.4	-	-
4-*O*-Acetyl-										
-vanillin 5c	191.1	135.2	110.8	152.0	144.9	123.4	124.8	56.1	168.4	20.7
-vanillin[90] 5c	190.73	135.16	110.87	151.85	144.85	123.29	124.42	55.98	168.05	20.52
-aldoxime **1c**	149.8	130.9	109.5	151.4	141.3	123.1	120.8	55.9	168.9	20.7
-oximyl **7c**	138.6	131.4	110.9	151.0	141.7	122.8	120.1	56.0	168.8	20.7
Dimers										
Aldehyde **5d**	191.6	125/128.6	109.6	148.6	151.0	125/128.6	128.1	56.5	-	-
Aldoxime **1d**	148.5	123.9	107.7	148.3	145.7	125.6	123.3	56.2	-	-

* DMSO-d_6



12c



12b

Table 5. Summary of ^{13}C-NMR shift data for the nitrile oxide-aldoxime adducts **12b**, **12c**

Di-4,4′-*O*-acetyl **12c**				OH, **12b**					
Carbon no.	δ_C predict.	δ_C $CDCl_3$	Δ_C $CDCl_3$	Carbon no.	δ_C predict.	δ_C C_6D_6	Δ_C C_6D_6	δ_C DMSO-d_6	Δ_C DMSO-d_6
1	84.4	101	16.6	1	84.4	101.7	17.3	101.1	16.7
2	138.2	134.3	-3.9	2	134	128	-6	127	-7
3	111.4	111.1	-0.3	3	112.4	109.9	-2.5	111.9	-0.5
4	156.9	151.2	-5.7	4	151.2	146.8	-4.4	148	-3.2
5	136.8	140.6	3.8	5	143.7	147.3	3.6	148.1	4.4
6	122.4	122.8	0.4	6	116.7	114.2	-2.5	115.7	-1
7	119.7	119.8	0.1	7	120.7	121.4	0.7	120.9	0.2
8	55.9	56.1	0.2	8	56.2	55	-1.2	56.2	0
9	169	169	0						
10	20.3	20.7	0.4						
11	164	159.2	-4.8	9	164	159.6	-4.4	160.2	-3.8
12	126.5	123.9	-2.6	10	122.3	117.9	-4.4	116.6	-5.7
13	110.7	111.5	0.8	11	111.7	109.8	-1.9	111.3	-0.4
14	157.2	151.2	-6	12	151.5	146.8	-4.7	148	-3.5
15	140.2	141.9	1.7	13	147	148.6	1.6	149.6	2.6
16	122.7	123.2	0.5	14	117	114.4	-2.6	115.9	-1.1
17	118.8	120.5	1.7	15	119.8	121.8	2	121.2	1.4
18	55.9	56.1	0.2	16	56.2	55	-1.2	56.2	0
19	169	169	0						
20	20.3	20.7	0.4						
Signals			20				16		16
Abs. Aver. error			2.5				3.8		3.2
Abs. aver. error excluding C-1			1.8				2.9		2.3
Abs. aver. error excluding C-1 & C-2			1.6				2.7		2.0

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