Tetrahedron 68 (2012) 8636–8644

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Viable and straightforward approach to the preparation of water soluble pyrazol-5-one derivatives through glycoconjugation

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ARTICLE INFO

Article history:
Received 24 February 2012
Received in revised form 22 June 2012
Accepted 23 July 2012
Available online 31 July 2012

Keywords:
Chromophore
Diazotization
Dye
Glycoconjugation
Lactose
Pyrazolone

ABSTRACT

The synthesis of water soluble pyrazol-5-one azo-dyes has been achieved. 6′-Aminolactosetriacetonide **9** was selected as the key building block to glyconjugate the pyrazole ring at its 3 position via a carboxamide or via a benzeneamide moiety in position 1 of the heterocycle. Diethyl-3-oxopentandionate **2** led eventually to a bicyclic compound, hampering the glyconjugation coupling process. The values of the molar extinction coefficients (ε) confirmed the tendency of pyrazolone azo dyes to exist in their tautomeric hydrazo form (especially in polar solvents); whereas the presence of substituents on the phenylazo group influenced the visible absorption maxima negligibly.

1. Introduction

Pyrazolones are well known heteroaromatic structures, which have been found use in many fields, since they exhibit biological and pharmacological properties[1] acting as anti–inflammatory agents,[2] anitipyretics[3] and inhibitors of protein kinases.[4] Pyrazolones have also been used as herbicides and bactericides,[5] as well as fungicides;[6] they possess the ability to enter into the regulatory mechanisms for the growth of vegetables,[5] although their eventual toxicity has been reported.[7] Recently, pyrazolone derivatives have been used as antiprion compounds.[8] These heterocycles are also known to be the building blocks of a class of dyes,[9,10] the most important commercial applications, of which are in the dyeing industry of natural and synthetic textiles, as well as in the manufacture of leather and rubber.[11] Pyrazol-5-one **1** is a heterocyclic species inclined to tautomerisation in polar solvents, displaying the corresponding pyrazol-5-ol **1a** with a certain character of aromaticity (Scheme 1).[12]

These interesting features of the pyrazolone ring motivated us to expand our recent developments about naturalized dyes[13] and start an investigation into this class of compounds. The purpose was to prepare pyrazolone-based moieties, bearing a carboxylic function to bond to a lactose unit derivative. This strategy could furnish new pyrazolone based derivatives, dyes for instance, circumventing their poor solubility in water and rendering them naturalized species.[13a]

1 **1a**

Scheme 1. Tautomerization of pyrazol-5-one **1**.

2. Results and discussion

We proceeded first to prepare a standard pyrazol-5-one, by reacting diethyl-3-oxopentandionate **2** with phenylhydrazine **3**. The process is known to initiate with a condensation reaction to a primary formed hydrazonic species, followed by the cyclization on the carboxylic function to end to the target compound **4**[14] in good yield. Then, having in mind the good reactivity of compound **4** in its 4 position towards electrophiles,[9] we subjected **4** to azo coupling with the diazonium salt **6a** prepared by diazotization of aniline **5a** under standard conditions:[15] obtaining the expected derivative **7**[16] in 81% yield. The following hydrolysis of the ethyl ester in position 3 of the pyrazolone, was carried out with NaOH in

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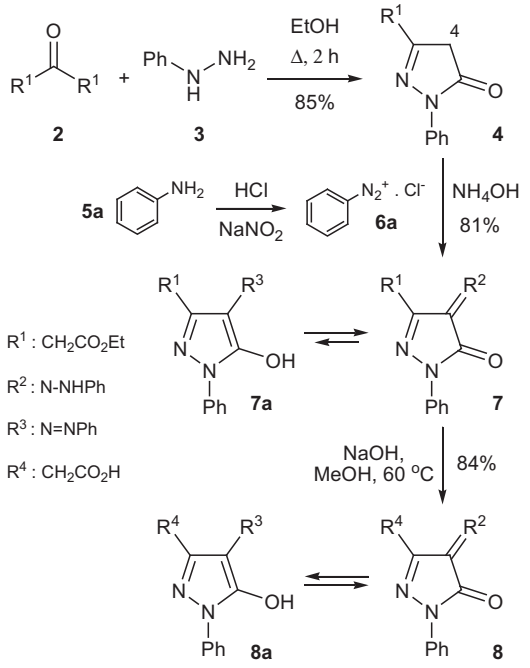
http://dx.doi.org/10.1016/j.tet.2012.07.074

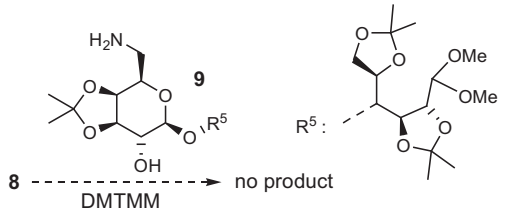
methanol and the corresponding acid **8** was obtained easily after acidification (Scheme 2).



Scheme 2. Synthesis of 4-azo-phenyl pyrazolone **8** from diethyl-3-oxopentandionate **2**.

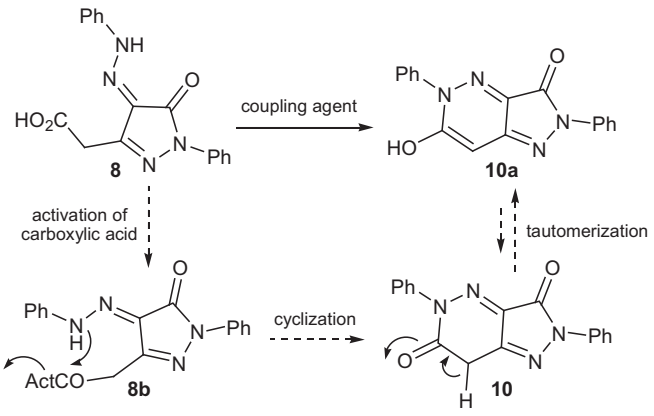
The ^{1}H NMR analysis of compound **7** in $CDCl_3$ did not show any spin resonance band attributable to the proton in α-position to the carbonyl of the pyrazolone ring. This evidence left the doubt of observing either the hydrazone form **7** or its enol tautomer **7a**.[17] The IR analysis clarified the issue, as the band for the C=O group of pyrazolone **7** was shown at 1658 cm^{-1},[17a] whilst no band attributable to the O–H bond of the enol form **7a** could be detected. In the case of compound **8** the concomitant presence of the corresponding enol tautomer **8a** could be acknowledged on the basis of the IR evidence. Beside the band at 1662 cm^{-1} due to the carbonyl group of **8** (cf. compound **7**) a band at 3435 cm^{-1} attributable to the O–H bond stretching of the enol form **8a**, was apparent (at variance with the case of **7**) and concomitant with the band of the O–H group of the carboxyl displaying a maximum centred at about 3170 cm^{-1}. This result indicated that compound **8** and its tautomer **8a** were both present in the solid state. It is reasonable to say that this tautomeric mixture would continue to exist in DMSO-d_6 solution, which was the solvent of choice to provide NMR analytical data.[17b] However, the ^{1}H- and ^{13}C NMR analysis could not indicate, which species would prevail at equilibrium.

At this point, the attempt to synthesize the glycoconjugated derivative of **8** was carried out according to our early reported procedure,[13a] employing 6′-aminolactosetriacetonide **9**[18] and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as the coupling agent (Scheme 3).[19]



Scheme 3. Attempted glyconjugation of **8** with **9**.

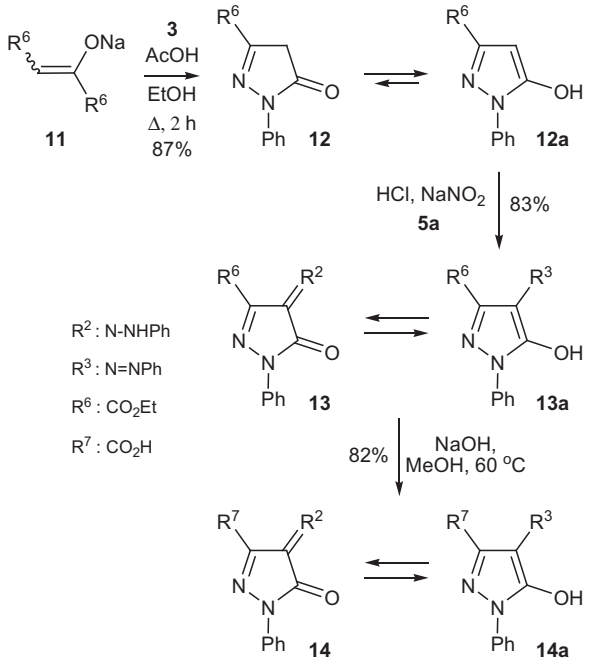
However, the reaction proved unsuccessful, since glycoconjugation was not achieved, whilst reactant **9** was fully recovered along with an unexpected product. Moving towards other types of carboxylic acid activators, such as *N*,*N*′-dicyclohexylcarbodiimide (DCC) *N*,*N*′-diisopropylcarbodiimide (DIC) or thionyl chloride[20] did not lead to glyconjugation of **8**–**9**. Yet, the same unexpected product was recovered. Eventually, rationalization of analytical data led us to conclude that 6-hydroxy-2,5-diphenyl-2*H*-pyrazolo [4,3-*c*]pyridazin-3(5*H*)-one **10a** had formed (Scheme 4).



Scheme 4. Proposed mechanism for the cyclization of **8** to bicycle **10**.

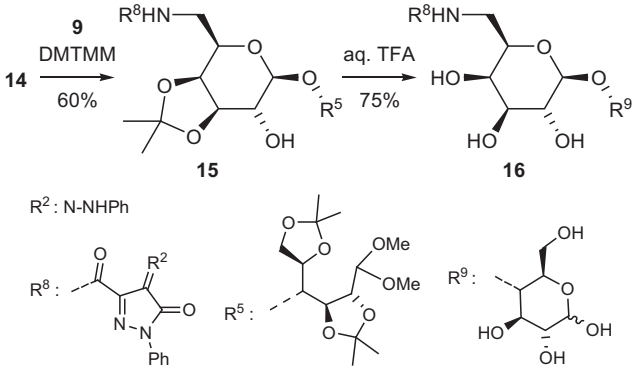
In particular, the ^{1}H NMR spectrum acquired in DMSO-d_6 as the unique deuterated solvent for this new product, did not show any spin resonance band attributable to the methylenic protons in position α to the carbonylic group of an eventual amide; whilst a singlet at 6.15 ppm was apparent for the enolic tautomer **10a**. Results from the mass spectrometric analysis were consistent with a cyclization reaction product: hence, we attributed the formation of **10** to an intramolecular process on the activated structure **8b**.[21] Therefore, we moved to a pyrazolone building block with a shorter arm in position 3, envisaging that cyclization would result not competitive with the glycoconjugation coupling. Thus, we used commercially available diethyloxalacetate sodium salt **11** in combination with **3** and acetic acid to achieve pyrazolone **12**,[22] where the ethylcarboxylate is directly bonded to the heterocycle. The following azo coupling step furnished the expected 4-azophenyl derivative **13**[17] and the successive hydrolysis permitted to obtain the carboxylic acid derivative **14**[23] (Scheme 5).

The ^{1}H NMR analysis of **12** in DMSO-d_6 clearly displayed a singlet at 5.98 ppm indicative for the enol species **12a**, whilst the spin resonance band assignable to the two protons in α-position to the carbonyl of the keto isomer **12** was not observed. This result was consistent with the observed band for the O–H bond at 3425 cm^{-1} in the IR analysis. Interestingly, no evidence was found for the C=O bond of the pyrazolone of **12**,[17a] indicating that enol **12a** might be the only detectable species at equilibrium. Also, the IR analysis pattern of compound **13** and **14** confirmed the concomitant presence of the enol tautomer **13a** and **14a**, respectively (cf. compound **8**). The acquisition of the ^{13}C NMR spectrum of **14** posed a practical issue, since acid **14** showed limited solubility even in DMSO-d_6. Furthermore, it was equally difficult to achieve full ^{13}C NMR characterization, since the C_{sp2} carbon of the carboxylic acid group could not be detected. Thus, it was decided to record the ^{13}C NMR spectrum of **14** by adding few drops of commercial conc. NH_4OH (28% w/w) to the NMR sample. The choice proved successful, since a 20 mg suspension of **14** in DMSO-d_6 became a homogeneous solution and more importantly, the carboxylic acid group could be observed at 163.4 ppm as the corresponding NH_4^+ carboxylate



Scheme 5. Synthesis of 4-azo-phenyl pyrazolone **14**.

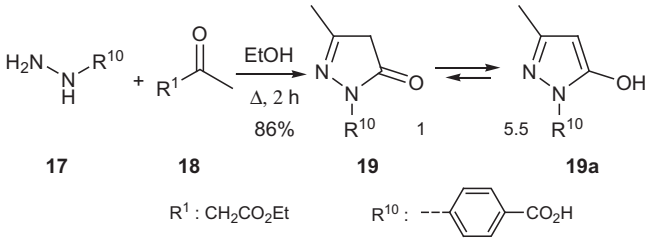
salt.[24] Next, DMTMM activation of acid **14** drove the glycoconjugation process with the amino partnering species **9** to product **15**. Finally, TFA mediated removal[13a] of the protecting groups resulted into the naturalized pyrazolone **16**, that exhibited a good solubility in water (Scheme 6).



Scheme 6. Glyconjugation of **14** with **9**.

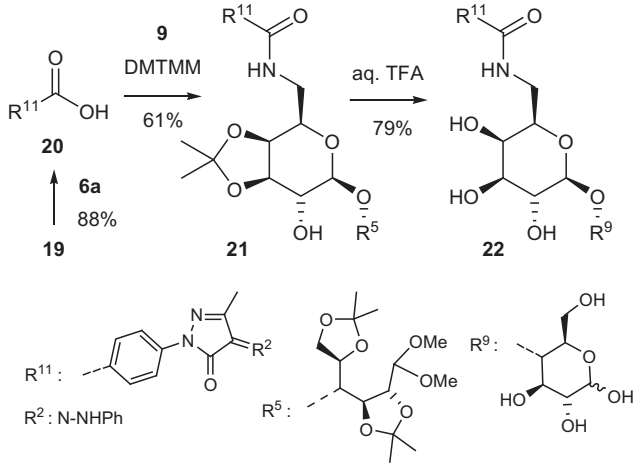
The success obtained in the naturalization of **14**, encouraged us to prepare a different compound, in which the carboxylic group was placed on the phenyl ring in position 1 of the heterocycle. Therefore, 4-hydrazinobenzoic acid **17** was chosen to reproduce the experimental procedure described for compound **12**: this time without adding acetic acid. Ethylacetoacetate **18** was the ketoester building block and this allowed to access pyrazolone **19**[25] in good yield (Scheme 7).

Interestingly, the ^{1}H NMR spectrum of **19** in DMSO-d_6 showed the signals of two species identified as tautomers **19** and **19a** in a 1:5.5 ratio, as measured by integrating the singlet at 5.2 ppm for the proton in position 4 of the enol form **19a** and the singlet at 3.2 ppm for the two protons in vicinal position to the carbonyl of the keto isomer **19**. On the contrary, the corresponding ^{13}C NMR spectrum was complex to rationalize, as the spin resonance bands for the enol species **19a** were broad. The addition of conc. NH_4OH



Scheme 7. Synthesis of pyrazolone **19**.

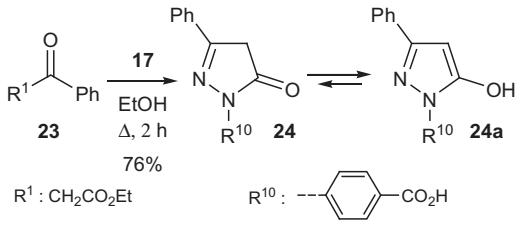
(cf. compound **14**) to the NMR sample shifted the equilibrium to the double salt form of **19a**, making the ^{13}C NMR characterization much easier to quote (see experimental). The following azo coupling reaction was carried out in water, adding a NaOH basic solution of **19** to an acidic solution of **6a**. Gratifyingly, product **20**[26] was obtained in 88% yield. This compound was coupled to **9** using DMTMM[18a] and product **21** was isolated in 73% yield after purification on silica gel. The fully deprotected naturalized pyrazolone **22** was prepared following the usual deprotection with TFA in good yield. Compound **22** showed good water solubility, at variance with pyrazolone **20** (Scheme 8).



Scheme 8. Elaboration of pyrazolone **19** to glyconjugated derivative **22**.

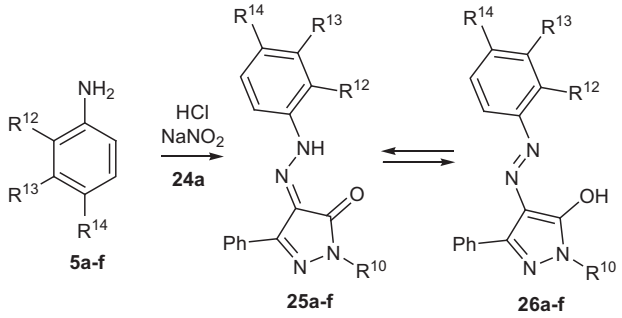
Compound **20** and its naturalized derivative **22** represent an interesting class of compounds, which may be expanded to a variety of species with different electronic absorption patterns and thus, considered potentially attractive for commercial applications. The chance to influence the electronic spectrum of compounds like **20** by decorating the phenyl ring in position 1, seems to appear misleading: this position of the pyrazolone ring behaves like a nodal point, impeding any efficient conjugation between the orbital π-system of the heterocycle and that of the phenyl ring.[27] Yet, substituents on an eventual aryl group in position 3 of the pyrazolone would not act as efficient auxochrome partners.[9] On the other hand, substituents on the phenyl ring directly bonded to the azo group promise more potential.[10] Hence, it was decided to embark on a preliminary study, synthesising a few derivatives of the new pyrazolone **24**, obtained by the condensation of **17** with ethyl–phenylacetoacetate **23** in 76% yield (Scheme 9).

In contrast to the ^{1}H NMR analysis of **19** under neutral conditions, the ^{1}H NMR spectrum of **24** in DMSO-d_6 showed the corresponding enol form **24a** only, characterised by a singlet at 6.04 ppm. In the IR analysis carried out in the solid state both species **24** and **24a** could be observed (see experimental) as previously discussed for compound **8** and **8a**. The further azo coupling



Scheme 9. Synthesis of pyrazolone **24**.

step was carried as described in Scheme 8 and products **25a**–**f** were isolated in discrete yields (75–95%) (Scheme 10).



a ; 75% , $R^{12} = R^{13} = R^{14} = H$
b : 84% , $R^{12} = R^{13} = H$, $R^{14} = NO_2$
c : 95% , $R^{12} = R^{14} = H$, $R^{13} = CF_3$
d : 90% , $R^{12} = R^{13} = H$, $R^{14} = COCH_3$
e : 94% , $R^{12} = H$, $R^{13} = R^{14} = F$
f : 89% , $R^{12} = R^{13} =$ -(HC=CH)-, $R^{14} = H$

Scheme 10. Diazotization of 5-hydroxy pyrazole **24a** with a series of anilines.

Yet again, the IR analyses of these compounds were consistent with those described earlier (cf. compound **8**) displaying the band for the C=O of the hydrazo species **25a**–**f** and the O–H bond of the enol tautomers **26a**–**f** (see experimental). The NMR characterization was achieved in DMSO-d_6: but the analytical data could not provide clear information about the tautomer composition of the mixture in solution.[17b] In the case of compound **25f** very low solubility was observed in DMSO-d_6; therefore, it was necessary to acquire the ^{13}C NMR spectrum, adding few drops of commercial conc. NH_4OH to the NMR sample (cf. compound **14**).

3. Spectrophotometric properties of azo-pyrazolone derivatives

The UV–vis spectra were registered in MeOH, since it allowed the comparison of the absorption maxima before and after glycoconjugation of the pyrazolones. Compound **8** exhibited an absorption maximum at 250 nm and 393 nm. The molar absorptivities were comparable, 17,060 and 17,358 M^{-1} cm^{-1}, respectively. When the carboxylic arm became shorter (compound **14**) a bathochromic and hyperchromic shift occurred. The two maxima were at 252 and 427 nm, while the molar absorptivities were 28,055 and 25,342 M^{-1} cm^{-1} (Fig. 1).

When the carboxylic acid was transformed to the amide of compound **15** the maximum in the visible moved slightly to 432 nm (ε=20,489 M^{-1} cm^{-1}). Considering compound **21** where the carboxyamide was placed on the phenyhydrazinic ring, the maximum in the visible shifted ipsochromically to 390 nm, with an ε as high as 24,236 M^{-1} cm^{-1} (Fig. 2).

As expected, glycoconjugation did not modify the spectra for **16** and **22** significantly, compared to those of the parent compounds (Fig. 3).

The series **25b**–**e** showed the interesting fluctuation of the maximum absorption around 400 nm: not too far from that

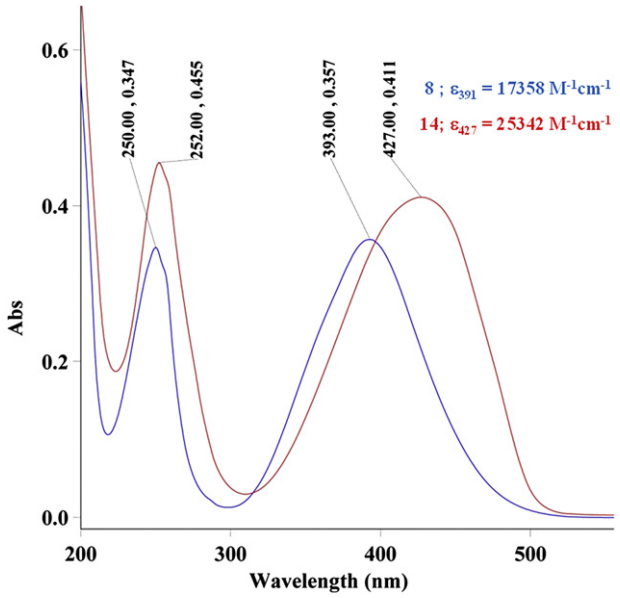


Fig. 1. UV–vis: **8** (l=0.1 cm, c=0.20 mM); **14** (l=0.1 cm, c=0.16 mM).

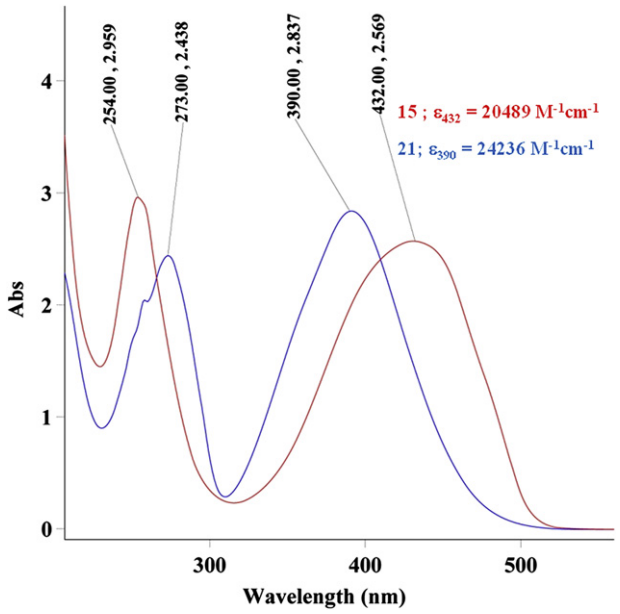


Fig. 2. UV–vis: **15** (l=1 cm, c=0.13 mM); **21** (l=1 cm, c=0.17 mM).

observed for compound **25a**, regardless of the EWG-character of the substituents on the phenylazo group (Table 1).[9]

These results indicated that pyrazolones having the 4 position in the form of a methylene likewise moiety, give rise to diazotized species, consisting mainly of the hydrazone tautomeric form **25a**–**e** (Scheme 10).[28] This aspect gives an explanation to the limited effects of the substituents on the phenyl-azo ring since in the prevalent tautomer the conjugation between the pyrazolone and the phenylazo substituent is not effective as expected. As a confirmation, the maximum absorption at 454 nm for structure **25f** may be explained by the increased steric hindrance of the naphtyl group, inducing the shift of the equilibrium towards a less congested structure like **26f**. In this case, the naphtyl group is allowed to overlap at its best to the π-orbital system of the N=N double bond, which in turn is conjugated to that of the pyrazolone ring.

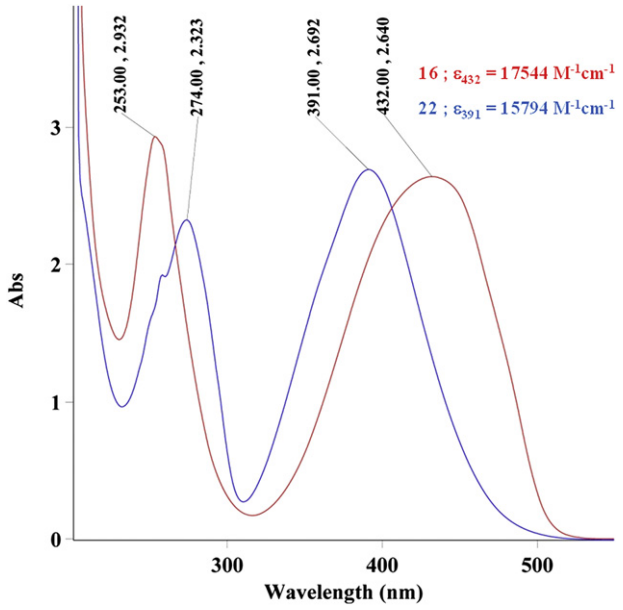


Fig. 3. UV–vis; **16** (l=1 cm, c=0.15 mM); **22** (l=1 cm, c=0.17 mM).

Table 1
Absorption maxima (λ_{max}) and molar absorptivities (ε) for the series **25a–f**

Products		λ_{max} (nm)	ε (M^{-1} cm^{-1})
25a	R^{12}=R^{13}=R^{14}=H	404	15.600
25b	R^{12}=R^{13}=H, R^{14}=NO_2	405	32.000
25c	R^{12}=R^{14}=H, R^{13}=CF_3	395	24.600
25d	R^{12}=R^{13}=H, R^{14}=$COCH_3$	408	21.600
25e	R^{12}=H, R^{13}=R^{14}=F	400	21.900
25f	R^{12}=R^{13}=–(CH=CH)–, R^{14}=H	454	17,600

4. Conclusions

A convenient approach to azo-pyrazolone based dyes has been reported and two compounds have been selected as representative examples for the naturalization process: either with the site of glycoconjugation directly linked to the pyrazolone ring or located on a side position (i.e., the aryl group in position 1 of the heterocycle). In both cases the results were appreciable, since the final glycocojugated species were quite water soluble. As this property of naturalised dyes depends upon the molecular weight of the lipophilic portion in the final molecule,[13a] the pyrazolones presented offer the opportunity to introduce two units of lactose, as we recently reported for commercial disperse dyes.[28] The pyrazolones here reported are yellow in nature: but the colour may be varied by an appropriate selection of substituents on the phenyl-azo group, generating naturalised dyes with tinctorial properties overlapping those of glycoconjugated disperse dyes.[13a] Currently, tinctorial tests of these new dyes are under investigation on synthetic and natural textiles. But the glyconjugation process here illustrated can be usefully applied to the pyrazolone-based derivatives in general, so that the property of water solubility can be easily reached with this kind of derivatives.

5. Experimental section

5.1. General

Commercially available reagents and solvents were purchased from *SigmaAldrich* and they were used directly. The notation PE refers to the petrol ether fraction boiling between 40 and 60 °C. Thin layer chromatography (TLC) analysis was performed using Fluka aluminium foils coated with 25 μm particle size silica gel matrix F_{254}. R_f values for compounds **4**, **7**, **8**, **12a**, **13**, **14**, **16**, **19**, **20**, **22**, **24a**, **25a–f** were quoted in a given solvent for characterization purposes only. TLC development involved either UV (254 and 366 nm) or visible light inspection, followed by either treatment with an acid solution of *p*-anisaldehyde or a basic solution of $KMnO_4$ and heating. Flash column chromatography was performed on Merck silica gel 60 (particle size 0.040–0.063 nm, 230–400 mesh ASTM) according to the procedure of Still.[29] Melting points were recorded on a Melting Point Apparatus SMP3-STUART SCIENTIFIC. Optical rotations were measured on a Jasco DIP-370 polarimeter using a 100 mm path-length cell at 589 nm. UV–vis spectra were recorded on a Cary-4000 Varian spectrophotometer, using either 0.1 or 1 cm quartz cuvettes. Infra-red spectra were recorded in a KBr disk on a Perkin Elmer-Spectrum BX FTIR system. Absorptions are quoted in wavenumbers (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 MHz ^{1}H (50.0 MHz ^{13}C) on a Varian Gemini spectrometer. Spin resonances are reported as chemical shifts (δ) in parts per million (ppm) and referenced to the residual peak as an internal standard of the solvent employed, as follows: $CDCl_3$ 7.27 ppm (^{1}H NMR), 77 ppm (^{13}C NMR, central band), DMSO-d_6 2.50 ppm (^{1}H NMR, central band), 39.5 ppm (^{13}C NMR, central band). Spin multiplicity is indicated by s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. Coupling constants *J* are reported in Hertz. Mass spectra were recorded on a ThermoScientific LCQ-Fleet mass spectrometer under electrospray ionisation (ESI, +c or −c technique). High Resolution Mass Spectra (HRMS) were recorded on a LTP-Orbitrap mass spectrometer from Thermo Electron Corporation under ESI (+c) technique. Mass spectrometric analysis is quoted in the *m*/*z* form. Elemental analyses were recorded on a Perkin Elmer 240 C Elemental Analyzer.

5.1.1. Synthesis of ethyl 2-(4,5-dihydro-5-oxo-1-phenyl-1H-pyrazol-3-yl)etanoate ***4***. In a 500 mL three neck round bottomed flask equipped with a condenser and a thermometer, phenylhydrazine **3** (10.8 g, 99.4 mmol, 1.1 equiv, 10.1 mL) was diluted in EtOH (140 mL) under magnetic stirring at 20 °C. Diethyl-3-oxopentandionate **2** (18.3 g, 90.4 mmol) was added and the resulting mixture was brought to reflux temperature for 2 h. The solution was then cooled to 20 °C and the solvent evaporated under reduced pressure. The residue was triturated with PE (200 mL) and kept under stirring for 6 h. The suspension was filtered in vacuo, obtaining the title compound (18.9 g, 85%) as pale yellow solid, mp 79–80 °C, lit.[14] 75–76 °C; R_f (10% EtOAc/CH2Cl2) 0.43; λ_{max} (ε) (MeOH) 244 nm (16,559 M^{-1} cm^{-1}); ν_{max} (KBr disk): 3140, *2972*, *2893*, *2794*, 1743, 1595, 1535, 1451, 1402, 1318, 1249, 1194 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.86–7.82 (2H, m, *Ar–H*), 7.43–7.36 (2H, m, *Ar–H*), 7.24–7.16 (1H, m, *Ar–H*), 4.23 (2H, q, *J* 7.2 Hz, $CO_2CH_2CH_3$), 3.64 (2H, s, CH_2CONPh), 3.58 (2H, s, $CH_2CO_2CH_2CH_3$), 1.31 (3H, t, *J* 7.2 Hz, $CO_2CH_2CH_3$); δ_C (50.3 MHz, $CDCl_3$) 170.5, 168.2, *152.7*, 137.7, 128.8 (2C), *125.2*, *118.8* (2C), *61.7*, *42.0*, *37.0*, *14.2*; ESI (*m*/*z*, −c) 245.2 $[M-1]^-$.

5.1.2. Synthesis of ethyl 2-[(4Z)-4-(2-phenylhydrazono)-4,5-dihydro-5-oxo-1-phenyl-1H-pyrazol-3-yl]etanoate ***7***. A 2.2 M solution of sodium nitrite (0.75 g, 10.8 mmol, 1.1 equiv) in water was added at 0 °C to aniline **5a** (*1.00* g, 10.8 mmol, 1.1 equiv, 1.0 mL) dissolved in 4.0 M HCl (7.4 mL). The acid solution was stirred for 10 min at 0 °C, after which a 0.5 M solution of compound **4** (*2.41* g, *9.80* mmol) in THF was added dropwise concomitantly with 1.3 M NH_4OH (14 mL). The resulting mixture was stirred for 0.5 h at 0 °C and then the suspension was filtered in vacuo. The solid was washed with H_2O (3×*20.0* mL) dried in air and further slurried in EtOAc:CH_2Cl_2:PE=1:1:8 (30 mL) for 3 h at 20 °C in a sealed flask.

The suspension was filtered under suction, to recover the title compound (*2.78* g, 81%) as orange solid, mp 120–121 °C, lit.[16] 118 °C; R_f (10% EtOAc/PE) 0.24; λ_{max} (ε) (MeOH) 250 (22,394), 393 nm (22,978) M^{-1} cm^{-1}; ν_{max} (KBr disk) 3062, 2973, 2933, 1733, 1659, 1594, 1560, 1545, 1486, 1347, 1268, 1184, 1150 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 8.00–7.96 (2H, m, *Ar–H*), 7.49–7.42 (6H, m, *Ar–H*), 7.28–7.20 (2H, m, *Ar–H*), 4.26 (2H, q, *J* 7.2 Hz, $CO_2CH_2CH_3$), 3.81 (2H, s, $CH_2CO_2CH_2CH_3$), 1.31 (3H, t, *J* 7.2 Hz, $CO_2CH_2CH_3$); δ_C (50.3 MHz, $CDCl_3$) 168.8, 157.5, 144.9, 140.8, 137.9, 129.6 (2C), 128.9 (2C), 127.4, 126.1, 125.3, 118.6 (2C), 115.9 (2C), 61.4, 32.9, 14.2; ESI (*m/z*, −c) 349.3 $[M-1]^-$.

5.1.3. Synthesis of 2-[(4Z)-4-(2-phenylhydrazono)-4,5-dihydro-5-oxo-1-phenyl-1H-pyrazol-3-yl]etanoic acid ***8***. In a 50 mL three neck round bottomed flask equipped with a condenser and a thermometer, ethyl ester **7** (*1.57* g, *4.50* mmol) was dissolved in MeOH (12 mL) and a solution of 1.2 M NaOH (5.3 mL) was added at 20 °C. The mixture was heated to 60 °C for 6 h and then it was cooled to 20 °C. A solution of 1.2 M HCl (5.5 mL) was added at 0 °C, obtaining the separation of an orange solid as a fairly granular precipitate. The suspension was filtered in vacuo and the solid was washed with an ice chilled solution of MeOH:H_2O=1:1 (2×20 mL) to dry in air as a fine orange powder (*1.22* g, 84%), mp 172–173 °C; [Found: C, 63.02; H, 4.29; N, 17.65. $C_{17}H_{14}N_4O_3$ requires 63.35; H, 4.38; N, 17.38%]; R_f (10% MeOH/CH_2Cl_2) 0.40; λ_{max} (ε) (MeOH) 250 (17,060), 393 nm (17,358 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3435 (*enol form* ***8a***), *3170*, 3073–2530 (br), 1708, 1662 (*keto form* ***8***), 1597, 1544, 1481, 1400, 1344, 1275, 1232, 1146 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 7.93–7.91 (2H, m, *Ar–H*), 7.63–7.60 (2H, m, *Ar–H*), 7.49–7.44 (4H, m, *Ar–H*), 7.26–7.22 (2H, m, *Ar–H*), 3.75 (2H, s, CH_2CO_2H); δ_C (50.3 MHz, DMSO-d_6) 170.2, *156.2*, 146.3, 141.3, 137.8, 129.6 (2C), 129.1 (2C), 126.8, 126.0, 125.0, 117.8 (2C), 116.4 (2C), 32.6; ESI (*m/z*, −c) 321.5 $[M-1]^-$.

5.1.4. Synthesis of 6-hydroxy-2,5-diphenyl-2H-pyrazolo[4,3-c]pyridazin-3(5H)-one ***10a***. In a 25 mL round bottomed flask acid **8** (*0.32* g, 1.0 mmol) was dissolved in THF (10 mL) at 20 °C. DIC (0.13 g, 1.0 mmol, 1.0 equiv, 0.15 mL) was added and the solution stirred overnight. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography (10% MeOH/EtOAc) isolating a dark brown solid (*0.10* g, 34%); [Found: C, 67.42; H, 4.02; N, 18.43. $C_{17}H_{12}N_4O_2$ requires C, 67.10; H, 3.97; N, 18.41%]; R_f (10% MeOH/EtOAc) 0.32; mp 140–142 °C; λ_{max} (ε) (MeOH) 272 nm (29,167 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3444 (br), 3064, 2857, 2754, 1705 (br), 1662–1589 (br), 1490, 1451, 1417, 1344, 1306, 1271, 1237, 1142, 1077 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 7.93 (2H, d, *J* 8.0 Hz, *Ar–H*), 7.61–7.42 (7H, m, *Ar–H*), 7.29–7.21 (1H, m, *Ar–H*), 6.29 (1H, s, *HC*=COH); δ_C (50.3 MHz, DMSO-d_6): 159.6, 156.0, 142.7, 142.0, 137.3, 133.8, 128.9 (2C), 128.5 (2C), 128.1, 126.1 (2C), 125.3, 118.7 (2C), 97.5; ESI (*m/z*, −c) 303.6 $[M-1]^-$.

5.1.5. Synthesis of ethyl 5-hydroxy-1-phenyl-1H-pyrazole-3-carboxylate ***12a***. In a 500 mL three neck round bottomed flask equipped with a condenser and a thermometer phenylhydrazine **3** (10.8 g, 99.4 mmol, 1.1 equiv, 10.1 mL) was diluted in EtOH (140 mL) and glacial AcOH (*5.95* g, *99.1* mmol, 1.1 equiv, 5.7 mL) was added dropwise at 20 °C. Diethyloxalacetate sodium salt **11** (*19.0* g, 90.4 mmol) was added portionwise under stirring and the resulting mixture was heated to reflux for 2 h. The solution was then cooled to 20 °C and the solvent evaporated in vacuo. The residue was partitioned between EtOAc (300 mL) and 0.5 M HCl (200 mL) and the organic phase was separated. The aqueous phase was extracted with EtOAc (50 mL) and the organic phases were combined, washed with brine (150 mL) and dried over Na_2SO_4. The suspension was filtered under suction and the filtrate was evaporated under reduced pressure, to afford the title compound (18.3 g, 87%) as pale yellow solid, mp 178–179 °C, lit.[22] 184–185 °C; R_f (30% PE/EtOAc) 0.47; λ_{max} (ε) (MeOH) 252 nm (16,976 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3425 (br), 3154, 3075, 2984, 2795, 1722, 1596, 1561, 1467, 1400, 1338, 1263, 1229, 1170 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 7.74 (2H, m, *Ar–H*), 7.55–7.47 (3H, m, *Ar–H*), 5.98 (1H, s, *HC*=COH), 4.28 (2H, q, *J* 7.0 Hz, $CO_2CH_2CH_3$), 1.29 (3H, t, *J* 7.0 Hz, $CO_2CH_2CH_3$); δ_C (50.3 MHz, DMSO-d_6) 161.8, 153.3, 142.1, 138.0, 129.0 (2C), 127.0, 122.2 (2C), 89.2, 60.3, 14.3; ESI (*m/z*, −c): 231.1 $[M-1]^-$.

5.1.6. Synthesis of (4Z)-ethyl-4-(2-phenylhydrazono)-4,5-dihydro-5-oxo-1-phenyl-1H-pyrazole-3-carboxylate ***13***. The synthesis of compound **13** was carried out on a 0.5 M THF solution of building block **12** (*9.80* mmol, *2.28* g) using aniline **5a** (1.0 g) 4 M HCl (7.4 mL) 2.2 M $NaNO_2$ (4.9 mL) and 1.3 M NH_4OH (14 mL) following the experimental procedure and work-up reported for compound **7**, isolating *the title compound* (2.75 g, 83%) as an air-dried orange solid, mp 148–150 °C, lit.[17] 151–152 °C; R_f (10% EtOAc/PE) 0.22; λ_{max} (ε) (MeOH) 253 (19,714), 410 nm (17,852 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3423 (*enol form* ***13a***), 3199, 2923, 1734, 1657 (*keto form* ***13***), 1602, 1548, 1501, 1412, 1270, 1246, 1201 and 1118 cm^{-1}; ^1H NMR δ (200 MHz, DMSO-d_6): 7.93–7.88 (2H, m, *Ar–H*), 7.69–7.65 (2H, m, *Ar–H*), 7.55–7.45 (4H, m, *Ar–H*), 7.39–7.28 (2H, m, *Ar–H*), 4.32 (2H, q, *J* 7.0 Hz, $CO_2CH_2CH_3$), 1.37 (3H, t, *J* 7.0 Hz, $CO_2CH_2CH_3$); δ_C (50.3 MHz, DMSO-d_6): 159.3, 156.1, 141.2, 138.6, 137.3, 129.7 (2C), 129.1 (2C), 126.8, 126.1, 124.8, 119.1 (2C), 117.0 (2C), 61.2, 14.1; ESI (*m/z*, +c): 337.1 $[M+1]^+$.

5.1.7. Synthesis of (4Z)-4-(2-phenylhydrazono)-4,5-dihydro-5-oxo-1-phenyl-1H-pyrazole-3-carboxylic acid ***14***. In a 50 mL three neck round bottomed flask equipped with a condenser and a thermometer ethyl ester **13** (*1.51* g, *4.50* mmol) was dissolved in MeOH (10 mL) at 20 °C and a 1.6 M solution of NaOH (4 mL) was added. The resulting mixture was heated to 60 °C for 6 h and then it was cooled to 20 °C. A solution of 1.6 M HCl (4.2 mL) was added dropwise at 0 °C, obtaining the precipitation of a yellow–orange solid. The suspension was filtered under suction and the solid was washed with an ice-chilled solution of MeOH:H_2O=1:1 (3×20 mL) and dried in oven at 55 °C (1.14 g, 82%), mp 232–233 °C, lit.[23] 231.5–232 °C; R_f (10% MeOH/CH2Cl2) 0.16; λ_{max} (ε) (MeOH) 252 (28,055), 427 nm (25,342 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3434 (*enol form* ***14a***), 3130–2370 (br), 1675, 1631 (*keto form* ***14***), 1597, 1564, 1482, 1367, 1307 cm^{-1}; δ_H (200 MHz, DMSO-d_6), 7.92–7.87 (2H, m, *Ar–H*), 7.55–7.44 (6H, m, *Ar–H*), 7.32–7.24 (2H, m, *Ar–H*); δ_C (50.3 MHz, DMSO-d_6+NH_4OH) 163.4, 162.2, 143.2, 140.9, 139.0, 130.2 (2C), 129.3 (2C), 126.0, 125.8, 125.4, 119.2 (2C), 116.7 (2C); ESI (*m/z*, −c) 307.2 $[M-1]^-$.

5.1.8. Synthesis of (4Z)-4-(2-phenylhydrazono)-N-{(3aS,4R,6S,7-R,7aR)-6-{(R)-[(4S,5R)-5-(dimethoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]-[(R)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}-tetrahydro-7-hydroxy-2,2-dimethyl-3aH-[1,3]dioxolo[4,5-c]pyran-4-yl}methyl-4,5-dihydro-5-oxo-1-phenyl-1H-pyrazole-3-carboxamide ***15***. In a 50 mL round bottomed flask azopyrazolone **14** (310 mg, 1.00 mmol) and 6′-aminolactosetriacetonide **9** (610 mg, *1.20* mmol, 1.2 equiv) were dissolved in THF (10 mL) and stirred for 5 h at 20 °C. DMTMM (290 mg, 1.05 mmol, 1.05 equiv) was added solid18a and the mixture was stirred overnight. The resulting suspension was partitioned between EtOAc (100 mL) and satd $NaHCO_3$ (50 mL) and then the organic phase was separated, washed with brine (2×50 mL) dried over Na_2SO_4 and filtered in vacuo. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography (40% EtOAc/CH_2Cl_2) to obtain the title compound (490 mg, 60%) as orange foam, mp 122–123 °C; [Found: C, 58.44; H, 6.24; N, 8.48. $C_{39}H_{51}N_5O_{13}$ requires C, 58.71; H, 6.44; N, 8.78]; R_f (40% EtOAc/CH_2Cl_2) 0.40; $[\alpha]_D^{20}$=−2.80 (*c* 1.12, $CHCl_3$); λ_{max} (ε) (MeOH) 254 (23,600), 432 nm (20,490 M^{-1} cm^{-1}); ν_{max} (KBr

disk) 3411 (br), 2985, 2934, 1703, 1594, 1551, 1479, 1454, 1380, 1371, 1303, 1215, 1145–1070 (br) cm^{-1}; δ_H (200 MHz, DMSO-d_6): 9.04 (1H, t, *J* 5.6 Hz, CO*NH*), 8.05–8.02 (2H, m, *Ar–H*), 7.50–7.46 (6H, m, *Ar–H*), 7.29–7.25 (2H, m, *Ar-H*), 5.32 (1H, d, *J* 5.2 Hz, CH*OH*), 4.40–4.29 (2H, m), 4.18–4.00 (7H, m), 3.91–3.81 (2H, m), 3.60–3.53 (2H, m), 3.27–3.21 (2H, m), 3.12 [6H, s, $(OCH_3)_2$], 1.43 (3H, s, CH_3 *acetonide*), 1.29 (3H, s, CH_3 *acetonide*), 1.24 (3H, s, CH_3 *acetonide*), 1.21 (3H, s, CH_3 *acetonide*), 1.16 (3H, s, CH_3 *acetonide*), 1.12 (3H, s, CH_3 *acetonide*); ^{13}C NMR δ_C (50.3 MHz, DMSO-d_6): 162.0, 161.3, 141.8, 138.4, 136.8, 130.5 (2C), 129.4 (2C), 126.7, 126.1, 125.2, 119.3 (2C), 116.7 (2C), 109.5, 109.2, 108.4, 105.5, 103.8, 79.8, 78.0, 76.8, 76.1, 75.8, 74.4, 73.0, 70.4, 66.2, 56.2, 54.7, 41.1, 28.6, 27.5, 27.0 (2C), 26.7, 25.8 ppm; ESI (*m*/*z*, +c): 820.4 $[M+Na]^+$.

*5.1.9. Synthesis of (4Z)-4-(2-phenylhydrazono)-N-{{(2R,3R,4S,5R,6S)-6-[(2R,3S,4R,5R)-tetrahydro-4,5,6-trihydroxy-2-(hydroxymethyl)-2H-pyran-3-yloxy]}-tetrahydro-3,4,5-trihydroxy-2H-pyran-2-yl}methyl-4,5-dihydro-5-oxo-1-phenyl-1H-pyrazole-3-carboxamide **16**.* In a 5 mL round bottomed flask glyconjugated derivative **15** (100 mg, 0.12 mmol) was dissolved in 90% aq TFA13a (1.5 mL). The dark yellow solution was stirred at 20 °C for 2 h and then it was diluted with toluene (50 mL) under vigorous stirring for 3 h. The resulting suspension was filtered and the solid was washed with toluene (3×10 mL) and dried in vacuo to recover the title compound (60 mg, 75%) as an indefinite mixture of α- and β-pyranosic anomers in the form of orange powder, mp 183–185 °C; R_f [1% H_2O/(15% MeOH/THF)] 0.58; $[\alpha]_D^{20}$=−26.00 (*c* 1.14, H_2O); λ_{max} (ε) (MeOH) 253 (19,485), 432 nm (17,544 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3390 (br), 2921 (br), 1651 (br), 1595, 1557, 1478, 1455, 1309, 1151 (br), 1052 (br) cm^{-1}; δ_H (200 MHz, DMSO-d_6): 9.08–9.04 (2H, m, CO*NH*), 8.05 (4H, d, *J* 7.6 Hz, *Ar–H*), 7.54–7.49 (12H, m, *Ar–H*), 7.32–7.26 (4H, m, *Ar–H*), 4.89–4.38 (2H, m), 4.34–4.24 (2H, m), 4.10–3.55 (24H, m), 3.46–3.15 (14H, m), 2.98–2.94 (2H, m); δ_C (50.3 MHz, DMSO-d_6) selected signals for the major isomer: 162.1, 161.4, 141.7, 138.4, 136.7, 130.4 (2C), 129.4 (2C), 126.7, 126.1, 125.0, 119.5 (2C), 116.9 (2C), 103.8, 97.1, 80.0, 75.2, 73.3, 72.8, 71.7, 70.8, 70.3, 69.1, 60.8, 40.7; HRMS (ESI): (*m*/*z*, +c): $[M+Na]^+$, found 654.2015. $C_{28}H_{33}N_5NaO_{12}$ requires 654.2023.

*5.1.10. Synthesis of 4-(4,5-dihydro-5-oxo-3-phenylpyrazol-1-yl)benzoic acid **19**.* In a 50 mL three neck round bottomed flask equipped with a condenser and a thermometer 4-hydrazynobenzoic acid **17** (*1.52* g, 10.0 mmol, 1.1 equiv) was suspended in EtOH (12 mL) at 20 °C. Ethylacetoacetate **18** (*1.18* g, *9.10* mmol, 1.0 equiv, 1.15 mL) was added and the resulting mixture was heated to reflux for 2.5 h. After cooling to 20 °C, the solvent was evaporated under reduced pressure and the residue was triturated in CH_2Cl_2 (20 mL). The suspension was stirred for 1 h and then it was filtered to obtain the title compound (*1.71* g, 86%) as off white powder, mp 280–281 °C, lit.[25] 283 °C; R_f (10% MeOH/CH_2Cl_2) 0.22; λ_{max} (ε) (MeOH) 282 nm (22,007 M^{-1} cm^{-1}); ν_{max} (KBr disk): 3400–2548 (br), 1692–1600 (br), 1513, 1414–1300 (br), 1200, 1150, 1110 cm^{-1}; δ_H (200 MHz, DMSO-d_6) *8.01–7.99* (*2H*, *m*, *Ar–H* ***19a*** and *2H*, m, *ArH* ***19***), *7.94–7.89* (*2H*, m, *Ar–H* ***19a*** and *2H*, m, *ArH*, ***19***), *5.40* [*1H*, br s, *HC*=*COH*(*NPh*) ***19a***], *3.74* [*2H*, br s, H_2CCO(*NPh*) ***19***], *2.14* (*3H*, s, CH_3 ***19a*** and *3H*, s, CH_3 ***19***); δ_C (50.3 MHz, DMSO-d_6+NH_4OH) 171.0, 164.4, 148.6, 142.9, 132.1 129.5 (2C), 116.9 (2C), 85.0, 15.1; ESI (*m*/*z*, −c): 217.4 $[M-1]^-$.

*5.1.11. Synthesis of 4-[(4Z)-4-(2-phenylhydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl]benzoic acid **20**.* A 1.25 M solution of sodium nitrite (0.17 g, 2.50 mmol, 1.07 equiv) in water was added at 0 °C to aniline **5a** (0.23 g, 2.50 mmol, 1.07 equiv) dissolved in 3.2 M HCl (2.9 mL). The acid solution was stirred for 10 min at 0 °C and *compound **19*** (0.51 g, 2.32 mmol, 1.0 equiv) dissolved in 0.7 M NaOH (7 mL) was introduced into the reaction flask. The resulting solution was stirred for 0.5 h and NaOAc 0.7 M (4.5 mL) was added, leading to the formation of a fine suspension, which was maintained at 0 °C for a further hour. Then, the whole was filtered under suction. The solid was washed with H_2O (3×10 mL) and dried in an oven at 70 °C to obtain the title compound (0.66 g, 88%) as dark orange solid, mp 273–275 °C, lit.[26] 277 °C; R_f (10% MeOH/CH_2Cl_2) 0.40; λ_{max} (ε) (MeOH) 274 (20,274), 391 nm (23,647 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3400–2400 (br), 1690 (br), 1600 (br), 1550–1480 (br), 1430 (br), 1370, 1340–1200 (br) cm^{-1}; δ_H (200 MHz, DMSO-d_6) 8.07–7.96 (4H, m, *Ar–H*), 7.62–7.58 (2H, m, *Ar–H*), 7.47–7.39 (2H, m, *Ar–H*), 7.24–7.17 (1H, m, *Ar–H*), 2.46 (3H, s, CH_3); δ_C (50.3 MHz, DMSO-d_6): 166.8, 156.9, 149.3, 141.3 (2C), 130.4 (2C), 129.5 (2C), 127.3, 126.4, 125.8, 116.9 (2C), 116.3 (2C), 11.6; ESI (*m*/*z*, −c): 321.6 $[M-1]^-$.

*5.1.12. Synthesis of 4-[(4Z)-4-(2-phenylhydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl]-N-{(3aS,4R,6S,7R,7aR)-6-{(R)-[(4S,5R)-5-(dimethoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]-[(R)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}-tetrahydro-7-hydroxy-2,2-dimethyl-3aH-[1,3]dioxolo[4,5-c]pyran-4-yl}methylbenzamide **21**.* The synthesis of compound **21** was carried out on a 1.0 mmol scale of building block **20** (0.32 g) using 6′-aminolactosetriacetonide **9** (610 mg, 1.2 mmol, 1.2 equiv) THF (10 mL) and DMTMM (290 mg, 1.05 mmol, 1.05 equiv) following the experimental, work-up and purification procedures reported for compound **15**, to isolate a foamy orange solid (0.51 g, 61%), mp 132–133 °C; [Found: C, 59.16; H, 6.90; N, 8.66. $C_{40}H_{53}N_5O_{13}$ requires C, 59.18; H, 6.58; N, 8.63]; R_f (40% EtOAc/CH_2Cl_2) 0.37; $[\alpha]_D^{20}$=+13.80 (*c* 1.32, $CHCl_3$); λ_{max} (ε) (MeOH) 258 (17,453), 273 (20,827), 390 nm (24,236 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3436 (br), 2986, 2935, 1662, 1552, 1503, 1370, 1342, 1268, 1219, 1152, 1071 (br); δ_H (200 MHz, $CDCl_3$) 8.30 (1H, t, *J* 4.8 Hz, CO*NH*), 8.05–7.95 (4H, m, *Ar–H*), 7.63 (2H, d, *J* 8.0 Hz, *Ar–H*), 7.49–7.44 (2H, m, *Ar–H*), 7.27–7.22 (1H, m, *Ar–H*), 5.30 (1H, d, *J* 5.2 Hz, *OH*), 4.51–4.39 (2H, m), *4.30* (1H, d, *J* 6.0 Hz), 4.22–3.89 (7H, m), 3.71–3.69 (1H, m), 3.52–3.48 (2H, m), 3.30–3.25 (2H, m), 3.25 [6H, s, $(OCH_3)_2$], 2.3 (3H, s, N=C-CH_3), 1.40 (3H, s, CH_3 acetonide), 1.33 [6H, s, (CH_3 acetonide)$_2$], 1.30 [6H, s, (CH_3 acetonide)$_2$], 1.2 (3H, s, CH_3 acetonide); δ_C (50.3 MHz, $CDCl_3$), 166.0, 156.8, 149.3, 141.3, 140.1, 130.2, 129.6 (2C), 128.4 (2C), 127.5, 125.8, 116.6 (2C), 116.3 (2C), 109.1, 108.6, 107.9, 105.1, 102.8, 79.2, 76.9, 76.3, 75.7, 74.9, 73.5, 72.5, 70.1, 65.7, *55.7*, *53.7*, 40.7, 28.1, 27.2, 26.6 (br, 2C), 26.2, 25.4, 11.6 ppm; ESI (*m*/*z*, −c): 810.85 $[M-1]^-$.

*5.1.13. Synthesis of 4-[(4Z)-4-(2-phenylhydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl]-N-{(2R,3R,4S,5R,6S)-6-[(2R,3S,4R,5R)-tetrahydro-4,5,6-trihydroxy-2-(hydroxymethyl)-2H-pyran-3-yloxy]-tetrahydro-3,4,5-trihydroxy-2H-pyran-2-yl}methylbenzamide **22**.* The synthesis of compound **22** was carried out on a 0.18 mmol scale of compound **21** (150 mg) using 90% aq TFA[13a] (2.3 mL) following the experimental procedure and work-up reported for compound **16**, to isolate the title compound (94 mg, 79%) as orange powder, mp 195–196 °C; R_f [1% H_2O/(15% MeOH/THF)] 0.29; $[\alpha]_D^{20}$=+27.10 (*c* 1.06, H_2O); λ_{max} (ε) (MeOH) 258 (10,114), 274 (13,629), 391 nm (15,793 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3369 (br), 2924 (br), 1659–1606 (br), 1553, 1506, 1343, 1273, 1153 (br), 1052 (br) cm^{-1}; δ_H (200 MHz, DMSO-d_6): 8.74–8.36 (2H, m, CO*NH*), 8.02–7.91 (8H, m, *Ar–H*), 7.63–7.60 (4H, d, *J* 8.8 Hz, *Ar–H*), 7.44–7.42 (4H, m, *Ar–H*), 7.22 (2H, t, *J* 7.8 Hz, *Ar–H*), 4.92–4.87 (1H, m), 4.76–4.72 (1H, m), 4.69–4.52 (2H, m), 4.47–4.30 (2H, m), 4.24–3.51 (20H, m), 3.43–3.06 (16H, m), 2.98–2.92 (2H, m), 2.30 [6H, s, $(CH_3)_2$]; δ_C (50.3 MHz, DMSO-d_6) selected signals for the major isomer: 166.7, 157.3, 149.7, 141.7, 140.5, 130.8, 130.1 (2C), 128.9 (2C), 128.0, 126.3, 117.2 (2C), 116.7 (2C), 104.2, 97.2, 80.7, 79.7, 75.3 (2C, br), 73.5, 73.1, 70.9, 69.0, 60.8, 43.3, 12.1; HRMS (ESI) (*m*/*z*, +c): $[M+Na]^+$, found 668.2170: $C_{29}H_{35}N_5Na\ O_{12}$ requires 668.2180.

*5.1.14. Synthesis of 4-(5-hydroxy-3-phenyl-1H-pyrazol-1-yl)benzoic acid **24a**.* A 100 mL three neck round bottomed flask equipped with

a condenser and a thermometer was charged with ethylbenzoylacetate **23** (*1.92* g, *10.0* mmol, 1 equiv) EtOH (40 mL) and 4-hydrazinobenzoic acid **17** (*1.67* g, *11.0* mmol, 1.1 equiv). The resulting mixture was heated to reflux for 1.5 h. Then, the solution was cooled to 20 °C and filtered in vacuo. The solid was washed with an ice chilled solution of H_2O:EtOH=2:1 (3 x 15 mL) to obtain the title compound (*2.13* g, 76%) as off white solid, mp 267–268 °C; [Found: C, 68.30; H, 4.03; N, 9.77. $C_{16}H_{12}N_2O_3$ requires C, 68.56; H, 4.32; N, 9.99]; R_f (10% MeOH/CH2Cl2) 0.35; λ_{max} (ε) (MeOH) 297 nm (28,200 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3420 (*enol form* ***24a***), 3080–2556 (br), 1688, 1656 (*keto form* ***24***), 1621, 1600, 1514, 1492, 1428, 1389, 1294, 1187 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 8.07–7.94 (4H, m, *Ar–H*), 7.86–7.81 (2H, m, *Ar–H*), 7.45–7.33 (3H, m, *Ar–H*), 6.04 [1H, s, *HC*=C(OH)]; δ_C (50.3 MHz, DMSO-d_6): 167.2, 155.0, 150.9, 142.5, 133.1, 130.7 (2C), 128.9 (2C), 128.5, 127.4, 125.5 (2C), 120.2 (2C), 86.0; ESI (*m*/*z*, –c): 297.49 $[M-1]^-$.

5.1.15. Synthesis of 4-[(4Z)-4-(2-phenylhydrazono)-4,5-dihydro-5-oxo-3-phenylpyrazol-1-yl]benzoic acid ***25a****.* A solution of aniline **5a** (0.18 g, *2.00* mmol, 1.1 equiv) in 1.4 M HCl (5 mL) was treated at 0 °C with 2.0 M $NaNO_2$ (0.14 g, *2.00* mmol, 1.1 equiv) in water. The resulting mixture was kept at 0 °C for 0.5 h and then an ice-chilled solution of compound ***24a*** (0.50 g, *1.80* mmol) in 0.5 M NaOH (5 mL) was added. The suspension was stirred for 1.5 h at 0 °C and the pH was adjusted to 5 with 0.5 M NaOH. The fine suspension was filtered and the solid washed with H_2O (3×10 mL) and EtOH (2×10 mL) to isolate the title compound (*0.52* g, 75%) as orange solid, mp 272–273 °C; [Found: C, 68.55; H, 3.92; N, 14.20. $C_{22}H_{16}N_4O_3$ requires C, 68.74; H, 4.20; N, 14.58]; R_f (10% MeOH/CH2Cl2) 0.53; λ_{max} (ε) (MeOH) 290 (31,200), 403 nm (15,600 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3417 (br) (*enol form* ***26a***), 3200–2543 (br), 1688, 1663 (*keto form* ***25a***), 1604, 1550, 1512, 1429, 1340, 1267 cm^{-1}; δ_H (200 MHz, DMSO-d_6): 8.15–8.11 (4H, m), 8.05–8.02 (2H, m), 7.60–7.58 (2H, m), 7.55–7.45 (5H, m), 7.27–7.24–6 (1H, m); δ_C (50.3 MHz, DMSO-d_6): 166.8, 157.3, 146.6, 141.2, 130.5 (2C), 130.0, 129.8 (2C), 129.7 (2C), 128.7 (2C), 127.2 (2C), 126.9, 126.2, 126.1, 117.2 (2C), 116.6 (2C); ESI (*m*/*z*, –c): 383.5 $[M-1]^-$.

5.1.16. Synthesis of 4-{(4Z)-4-[2-(4-nitrophenyl)hydrazono]-4,5-dihydro-5-oxo-3-phenylpyrazol-1-yl}benzoic acid ***25b****.* The synthesis of compound **25b** was carried out on a *1.00* mmol scale of building block ***24a*** (0.28 g) dissolved in 0.5 M NaOH (2.77 mL) using 4-nitroaniline **5b** (0.15 g) 1.4 M HCl (2.77 mL) and 2.0 M $NaNO_2$ (0.55 mL) following the experimental procedure and work-up reported for compound **25a**, to isolate the title compound (0.36 g, 84%) as dark brown solid, mp 300–301 °C; [Found: C, 61.45; H, 3.57; N, 16.38. $C_{22}H_{15}N_5O_5$ requires C, 61.54; H, 3.52; N, 16.31]; R_f (10% MeOH/CH2Cl2) 0.51; λ_{max} (ε) (MeOH) 289 (27,800), 405 nm (32,000 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3426 (br) (*enol form* ***26b***), 3180–2538 (br), 1697, 1666 (*keto form* ***25b***), 1606, 1555, 1507, 1430, 1340, 1245, 1163, 1109 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 8.33 (2H, d, *J* 6.2 Hz), 8.20–8.14 (4H, m), 8.09–8.05 (2H, m), 7.86 (2H, d, *J* 6.2 Hz), 7.62–7.55 (3H, m); δ_C (50.3 MHz, DMSO-d_6): 170.5, 161.6, 154.0, 148.3, 143.3, 141.4, 134.4, 134.2, 129.8 (2C), 128.4–128.3 (5C, br), 126.4, 125.3 (2C), 120.3 (2C), 117.1 (2C); ESI (*m*/*z*, –c): 428.14 $[M-1]^-$.

5.1.17. Synthesis of 4-{(4Z)-4-{2-[3-(trifluoromethyl)phenyl]hydrazono}-4,5-dihydro-5-oxo-3-phenylpyrazol-1-yl}benzoic acid ***25c****.* The synthesis of compound **25c** was carried out on a 1.0 mmol scale of building block ***24a*** (0.28 g) dissolved in 0.5 M NaOH (2.77 mL) using using 3-trifluomethylaniline **5c** (0.18 g) 1.4 M HCl (2.77 mL) and 2.0 M $NaNO_2$ (0.55 mL) following the experimental procedure and work-up reported for compound **25a**, to isolate the title compound (0.43 g, 95%) as dark yellow solid, mp 270–271 °C; [Found: C, 60.97; H, 3.44; N, 12.46. $C_{23}H_{15}F_3N_4O_3$ requires C, 61.06; H, 3.34; N, 12.38]; R_f (10% MeOH/CH2Cl2) 0.33; λ_{max} (ε) (MeOH) 290 (30,200), 395 nm (24,600 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3415 (br) (*enol form* ***26c***), 3300–2525 (br), 1686, 1665 (*keto form* ***25c***), 1604, 1553, 1462, 1424, 1329, 1281 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 8.10–7.86 (8H, m), 7.68–7.64 (1H, m), 7.54–7.49 (4H, m); δ_C (50.3 MHz, DMSO-d_6): 166.7, 156.7, 146.7, 142.3, 141.0, 130.8, 130.4 (2C), 130.2 (1C, q, $^2J_{C,F}$ 31.8 Hz, *Ar–C*$_q$), 130.1, 129.5, 128.6 (2C), 127.2 (3C), 126.8, 124.2 (1C, q, $^1J_{C,F}$ 271.0 Hz, *CF*$_3$), 121.8 (br), 119.9, 117.0 (2C), 113.4 (br); ESI (*m*/*z*, –c): 451.07 $[M-1]^-$.

5.1.18. Synthesis of 4-{(4Z)-4-[2-(4-acetylphenyl)hydrazono]-4,5-dihydro-5-oxo-3-phenylpyrazol-1-y}benzoic acid ***25d****.* The synthesis of compound **25d** was carried out on a 1.0 mmol scale of building block ***24a*** (0.28 g) dissolved in 0.5 M NaOH (2.77 mL) using 1-(4-aminophenyl)ethanone **5d** (0.15 g) 1.4 M HCl (2.77 mL) and 2.0 M $NaNO_2$ (0.55 mL) following the experimental procedure and work-up reported for compound **25a**, to isolate the title compound (0.38 g, 90%) as dark yellow solid, mp 274–275 °C; [Found: C, 67.53; H, 4.30; N, 12.80. $C_{24}H_{18}N_4O_4$ requires C, 67.60; H, 4.25; N, 13.14]; R_f (10% MeOH/CH2Cl2) 0.44; λ_{max} (ε) (MeOH) 292 (26,800), 407 nm (21,600 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3434 (br) (*enol form* ***26d***), 3029–2515 (br), 1715, 1675 (*keto form* ***25d***), 1603, 1547, 1250, 1166 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 8.13–7.98 (8H, m), 7.70–7.66 (2H, m), 7.53–7.51 (3H, m), 2.53 (3H, s, CO*CH*$_3$); δ_C (50.3 MHz, DMSO-d_6): 199.2, 172.2, 159.7, 155.1, 149.1, 142.0, 134.4, 134.2, 134.0, 130.4 (4C), 128.9 (4C), 125.7, 125.1, 120.8 (2C), 118.5 (2C), 27.1; ESI (*m*/*z*, –c): 425.12 $[M-1]^-$.

5.1.19. Synthesis of 4-{(4Z)-4-[2-(3,4-difluorophenyl)hydrazono]-4,5-dihydro-5-oxo-3-phenylpyrazol-1-yl}benzoic acid ***25e****.* The synthesis of compound **25e** was carried out on a 1.0 mmol scale of building block ***24a*** (0.28 g) dissolved in 0.5 M NaOH (2.77 mL) using 3,4-difluoroaniline **5e** (0.14 g) 1.4 M HCl (2.77 mL) and 2.0 M $NaNO_2$ (0.55 mL) following the experimental procedure and work-up reported for compound **25a**, to isolate the title compound (0.39 g, 94%) as dark brown solid, mp 267–269 °C; [Found: C, 62.75; H, 3.47; N, 13.11. $C_{24}H_{14}F_2N_4O_3$ requires C, 62.86; H, 3.36; N, 13.33]; R_f (10% MeOH/CH2Cl2) 0.36; λ_{max} (ε) (MeOH) 290 (27,500), 400 nm (21,900 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3395 (br) (*enol form* ***26e***), 3190–2510 (br), 1689, 1661 (*keto form* ***25e***), 1605, 1555, 1504 (br), 1426, 1337, 1253, 1174 cm^{-1}; δ_H (200 MHz, DMSO-d_6) 8.18–8.10 (4H, m), 8.03–7.98 (2H, m), 7.71–7.60 (1H, m), 7.57–7.45 (5H, m); δ_C (50.3 MHz, DMSO-d_6): 166.7, 156.3, 149.7 (1C, dd, $^1J_{C,F}$ 245 and $^2J_{C,F}$ 13.7 Hz, *Ar–C*$_q$*F*), 147.2 (1C, dd, $^1J_{C,F}$ 245 and $^2J_{C,F}$ 12.8 Hz, *Ar–C*$_q$*F*), 146.8, 141.5, 141.0 (br), 130.3 (3C), 129.5, 128.4 (2C), 127.2 (2C), 126.3, 125.9, 118.1 (1C, d, $^2J_{C,F}$ 18.6 Hz, *Ar–CH*), 116.9 (2C), 113.7 (br), 106.2 (1C, d, $^2J_{C,F}$ 21.1 Hz, *Ar–CH*); ESI (*m*/*z*, –c): 419.14 $[M-1]^-$.

5.1.20. Synthesis of 4-{(4Z)-4-[2-(naphthalen-1-yl)hydrazono]-4,5-dihydro-5-oxo-3-phenylpyrazol-1-yl}benzoic acid ***25f****.* The synthesis of compound **25f** was carried out on a 1.0 mmol scale of building block ***24a*** (0.28 g) dissolved in 0.5 M NaOH (2.77 mL) using 1-naphtylamine **5f** (0.16 g) 1.4 M HCl (2.77 mL) and 2.0 M $NaNO_2$ (0.55 mL) following the experimental procedure and work-up reported for compound **25a**, to isolate the title compound (0.39 g, 89%) as dark brown solid, mp 276–277 °C; [Found: C, 71.79; H, 3.81; N, 12.50. $C_{26}H_{18}N_4O_3$ requires C, 71.88; H, 4.18; N, 12.90]; R_f (10% MeOH/CH2Cl2) 0.39; λ_{max} (ε) (MeOH) 207 (38,800) 290 (23,100), 454 nm (17,600 M^{-1} cm^{-1}); ν_{max} (KBr disk) 3451 (br) (*enol form* ***26f***), 3180–2533 (br), 1685, 1658 (*keto form* ***25f***), 1604, 1560, 1512, 1425, 1284, 1256, 1175 cm^{-1}; δ_H (200 MHz, DMSO-d_6): 8.27–8.21 (4H, m), 8.11–8.02 (4H, m), 7.98–7.86 (2H, m), 7.80–7.75 (1H, m), 7.70–7.55 (5H, m); δ_C (50.3 MHz, DMSO-d_6+NH_4OH) 171.7, 157.0, 151.1, 147.8 142.2, 135.3, 134.7, 134.0, 130.4, 130.2 (2C), 128.8 (2C),

128.6 (2C), 128.4, 128.1, 126.9, 126.6, 125.9, 124.9, 124.7, 118.1 (2C), 110.3, 79.5 ppm; ESI (m/z, −c): 433.53 $[M-1]^-$.

Acknowledgements

The authors are indebted to the Ente Cassa di Risparmio di Firenze, who granted the present research and funded the purchase of the Varian UV–Vis spectrophotometer and the mass spectrometer ThermoFisher LCQ fleet ion trap instrument.

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