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Solid Support Synthesis of Highly Functionalized Pyrazoles and Isoxazoles; Scaffolds for Molecular Diversity

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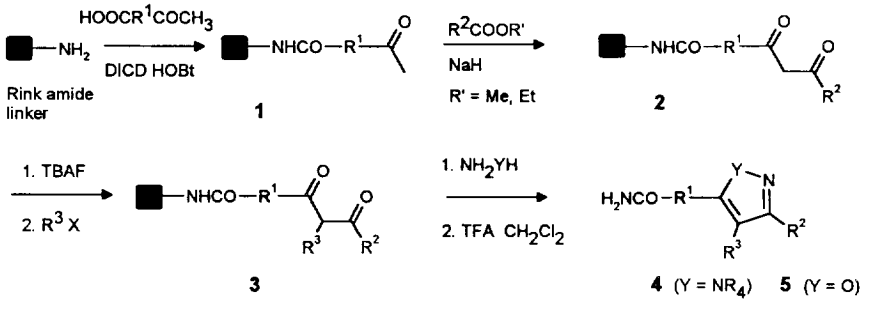
Abstract: A *scope and limitations* study on a 4 steps reaction sequence including a Claisen condensation, an α-alkylation, and a cyclization of a β-diketone with monosubstituted hydrazines for the generation of variably substituted pyrazoles and isoxazoles on solid phase is described. The ample choice of reactants compatible with the reaction scheme points out its potential in combinatorial chemistry.

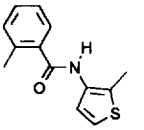
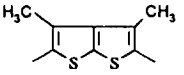
The synthesis of combinatorial compound libraries is rapidly taking on the role of a powerful component within modern lead finding processes that aim at the identification of compounds with novel target activities of interest[1]. In the drug discovery context, the ability to synthesize small organic molecules with high yield on a solid support has a definite strategic relevance. It facilitates the preparation of compound arrays in multiple parallel syntheses and enables the application of combinatorial methods for the synthesis of large libraries suitable for systematic evaluations in biochemical or biological test systems. In view of the expected biostability and bioavailability, small organics (e.g. heterocycles) rather than chain-like biooligomers are more attractive leads for subsequent medicinal chemistry efforts.

Scheme 1:



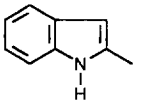
Here we report a scope and limitation study on a 4 steps reaction sequence on solid phase, suitable for the generation of molecular diversity on small heterocycles of the pyrazole and isoxazole type (Scheme 1). For each reaction, suitable conditions on solid phase were worked out and a variety of more or less reactive agents (building blocks) was utilized in an effort to grasp the system's breadth of applicability. To that end, Tables 1 - 4 list the residues of the reagents and products, of which the conversions on solid phase were monitored by LCMS of starting materials and product samples, cleaved from the solid support into solution[2]. Our investigations therefore concentrated, at this stage, on the performance of discrete steps within an overall reaction scheme that we intend to exploit by the combinatorial approaches of the *split and mix* concept[3].

Table 1. Coupling procedure to acetyl compounds **1a-c**

	R^1	loading[1]		R^1	loading[1]
1a	4-Ph	424	**1c**		–[2]
1b		410			

1) resin capacity [μmol g^{-1}]
2) intramolecular ring closure reaction

Table 2. Claisen condensation to β-diketone **2a-k** (R^1 = 4-Ph)

	R^2	yield[1]		R^2	yield[1]
2a	Ph	100%	**2h**		100%
2b	4-$CH_3OC_6H_4$	100%	**2i**		95%
2c	4-ClC_6H_3(2-NO_2)	–[2]			
2d	4-ClC_6H_3(2-Cl)	100%	**2j**		100%
2e	4-$CH_3OOCC_6H_4$	100%			
2f	4-NCC_6H_4	100%	**2k**		–[4]
2g	CH_3	–[3]			

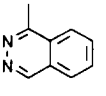
1) by HPLC
2) is prone to reduction
3) multicomponent mixture
4) formation of indolyl anion

Table 3. α-Alkylation of diketone **2a** (R^1 = 4-Ph, R^2 = Ph)

	R^3	X	yield[1]		R^3	X	yield[1]
3a	Et	I	90 %	**3d**	$NCCH_2$	I	60 %
3b	Bz	Br	0 %	**3e**	$PHCOCH_2$	Br	65 %
3c	$EtOOCCH_2$	Br	90 %	**3f**	CH_2=$CHCH_2$	Br	90 %

1) by HPLC

Table 4. Formation of pyrazoles **4a-d** (R^1 = 4-Ph, R^2 = Ph, R^3 = Et) and isoxazole **5a** (R^1 = 4-Ph, R^2 = Ph, R^3 = Et)

	Y	yield[1]		Y	yield[1]
4a	NH	100 %	**5a**	O	100 %
4b	NCH_3	100 %			
4c	NPh	100 %	Phz :		
4d	NPhz	20 %			

1) by HPLC of regioisomers

In the first step the solid support is loaded with the R^1 component bearing the acetyl function. Its carbonyl group is activated by standard methods and anchored to the acid labile Rink amide linker on polystyrene[4,5]. We observed quantitative transformations within 1 hour, unless ortho substituted bifunctional derivatives like o-acetophenone and acetylphthalanilidic acid were used, which undergo ring closure side reactions[6].

For the Claisen condensation, optimization of the reaction protocol with the prototypic aromatic ester ethyl benzoate identified conditions, which ensure that also deactivated benzoates and heteroaromatic carboxylic esters condense without appreciable formation of side products[7]. As expected, carboxylic esters with α-hydrogens (**2g**) are unsuited, and also weakly acidic heteroaromatic compounds (**2k**) cannot be applied. The same is the case for nitro derivatives which are prone to reduction. Noticeably, the series of successful conversions to the diketone included a deactivated ester (**2b**), as well as a bifunctional building block and a component with an additional electrophilic center (**2e** and **2f** resp.), although in the latter cases compatibility with the following reaction steps is not granted.

With regards to the α-alkylation step, we obtained best results in the presence of TBAF[8], which has the function to shield the oxygen atoms of the β-dicarbonyl intermediate, hence inhibiting O-alkylation as a side reaction and furthermore increasing the nucleophilicity of the compound. Water traces are detrimental to the yield, which otherwise lies around 90% of the C-monoalkylated product. To expand the diversity, we also experimented with alkylating agents other than the simple alkyl iodides described in analogous solution chemistry[9]. Ethyl bromoacetate and allyl bromide reacted without side reactions. With iodoacetonitrile 35% of starting material was observed and bromoacetophenone did not convert cleanly. The failure with benzyl bromide was rather unexpected. The alkylation step is incompatible with the presence of acid or basic heteroaromatic R^1 and R^2 residues: with the phenyl pyridine diketone **2j** several side products were observed upon alkylation with all the listed alkylating agents. Naturally, dispensing with the alkylation altogether enables to broaden the choice of building blocks for the previous Claisen condensation by allowing e.g. the inclusion of N-heterocyclic esters as an alternative source of diversity.

Ring closure to form a heterocyclic scaffold was tested successfully with hydrazines **4a-d** and hydroxylamine (**5a**)[10]. With the non-alkylated intermediate **2a** a faster cyclization kinetics than with **3a** was observed. N-mono-substituted reagents were expected to yield regioisomers with equal efficiency, unless the intermediates would bear large differences of steric and electronic properties at the R^1 and R^2 sites.

During the validation process we isolated both regioisomers of model compound **4d**[11]. In this instance we intentionally explored the limits by chosing a difficult case, since hydralazine (for electronic and steric reasons) was predicted to have a relatively weak tendency to cyclize. In fact, after 1 day, only traces of the regioisomers **4d** were detectable, and it took 4 days to obtain a level of 20 % conversion, in the presence of the unsubstituted analog **4a** originating from the far more reactive impurity **2a** (the non-alkylated diketone precursor).

The collected data on the conversion rates of a variety of reactants within our proposed synthetic scheme provide an information basis sufficient for the planning of combinatorial libraries along different lines of strategies. Decisions can be made on which types of building blocks to include for diversity generation and whether to skip a reaction step in favour of a broader choice of compatible residues or simplified reaction conditions. Moreover, the general utility of the approach could be exploited to form additional ring types (e.g. pyrimidines from amidines) if the diketone intermediates were subjected to cyclization with other reagents bearing two nucleophilic centers.

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References and Notes

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2. Cleavage from the support was done by diluted TFA[5]. HPLC analytical separation was achieved using a reverse phase nucleosil C18 5μ 250 mm x 4.6 mm column, 215 nm, 10-90% CH_3CN/0.1% TFA over 30 min, 1 ml/min. A part of the eluate (split 1:25) was introduced into a Quattro-BQ mass spectrometer (VG Biotech, Altrincham, England), operated at a source temperature of 60°C and a cone voltage of 50 V, via an electrospray interface (EI). The mass range from 100 to 800 Dalton was scanned in 4 seconds.
3. (a) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Abstr. 14th Int. Congr. Biochem., Prague* **1988**, *5*, 47. (b) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 487.
4. *Deprotection:* 4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)phenoxy resin (Rink amide resin) was subjected to repeated washes with 20% piperidine/DMF until no UV absorption from Fmoc was detected in the eluate. *Coupling procedure:* The NH_2-linker group was acylated with 0.3M-solution of acetyl carboxylic acid (3eq) at RT (preactivation 40min with 3.3eq DICD and 3.3eq HOBt) until the Kaiser test (Kaiser, E. et al. *Anal. Biochem.* **1970**, *34*, 595) was negative.
5. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
6. Nishio T.; Yamamoto H. *J. Heterocycl. Chem.* **1995**, *32*, 883.
7. *Claisen condensation:* 50 mg (22.5 μmol) resin were suspended in a solution of 675 μmol carboxylic ester in 670 μl DMA. Under inert gas 18 mg (450 μmol) of sodium hydride (60%) was added and the reaction mixture was well shaken for 1h at 90 °C. The resin was filtered, washed (30% v/v acetic acid / H_2O, DMA, DMSO, and i-PrOH), and dried under reduced pressure.
8. *Alkylation:* 20 mg (8.6 μmol) of this resin was treated with 86 μl 1M TBAF in THF for 2h at room temperature. After addition of 150 μl of a 2.5M solution of the appropriate alkylating agent, the reaction was continued for another 2h. The resin was filtered off and washed well with CH_2Cl_2 and THF.
9. Clark, J. H.; Miller, J. M.; *J. Chem Soc., Perkin Trans. I* **1977**, 1743
10. *Cyclization:* The resulting resin was heated with 500 μl of a 2.5 M solution of hydrazines or hydroxyl amine (HCl was neutralised by NEt_3) in DMA at 80 °C for 24 h. For final cleavage conditions see lit.[5].
11. **1a** was formed from 1.50 g Rink amide resin by the standard coupling protocol; 100% by HPLC, 10.2 min, MS (EI) m/z 163 (M^+). Treatment of resin **1a** with 0.51g (3.4 mmol) PhCOOEt, 0.14 g (3.4 mmol) NaH (60% dispersion), and 10.5 ml DMA provided **2a**; 95% by HPLC, 26.3 min, MS (EI) m/z 267 (M^+). Conversion of resin **2a** with 6.75 ml (6.75 mmol) 1M TBAF in THF, 5.68 ml CH_2Cl_2, 2.11 g (13.5 mmol) EtI afforded **3a** (75% by HPLC, 22.7 min, MS (EI) m/z 294 (M^+). Heating resin **3a** 4 days under reflux with 1.09 g (6.75 mmol) hydralazine and 67 mg (0.67 mmol) acetylacetone in EtOH gave $\mathbf{4d_1}$ (10% by HPLC, 25.3 min) and $\mathbf{4d_2}$ (10% by HPLC, 26.3 min) after cleavage with 20% v/v TFA/CH_2Cl_2. HPLC preparative separation was achieved using a reverse phase nucleosil C18 5μ 20 mm x 250 mm column, 215 nm, 10-90% CH_3CN/0.1% TFA over 90 min, 15 ml/min. $\mathbf{4d_1}$ ^{1}H-NMR (DMSO-d_6) δ 9.72 (s, 1H), 8.29 (m, 1H), 8.11 (m, 4H), 8.02 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H) 7.46 (bs, 1H), 7.32 (m, 2H), 7.24 (m, 1H), 2.77 (q, J = 7.4 Hz, 2H), 1.06 (t, J = 7.4 Hz, 3H), MS (EI) m/z 420 (M^+). $\mathbf{4d_2}$ ^{1}H-NMR (DMSO-d_6) δ 9.71 (s, 1H), 8.29 (m, 1H), 8.20 (m, 1H), 8.15 (m, 2H), 7.99 (bs, 1H), 7.82 (m, 4H), 7.51 (m, 4H), 7.42 (bs, 1H), 7.31 (d, J = 8.5 Hz, 2H), MS (EI) m/z 420 (M^+).

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