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Solid-phase versus solution synthesis of asymmetrically disubstituted furazano[3,4-*b*]pyrazines

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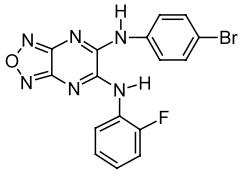
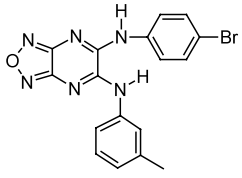
Abstract—Herein we describe a straightforward solid-phase synthesis directed towards the preparation of families of asymmetrically disubstituted furazano[3,4-*b*]pyrazines by stepwise displacement of the two chlorine atoms in 5,6-dichlorofurazano[3,4-*b*]pyrazine by nucleophiles. This synthesis has avoided selectivity problems found in solution chemistry. © 2002 Published by Elsevier Science Ltd.

The furazano[3,4-*b*]pyrazine heterocyclic system has attracted much attention from researchers as an example of energy-rich polynitrogenated compounds.[1] In addition, it has been found that compounds structurally related to furazano[3,4-*b*]pyrazines have also shown biological activity in the field of antimicrobials,[2] herbicides and plant growth regulators.[3]

As a result of our High Throughput Screening activities aimed at finding new antibacterials active against *S. aureus* and *E. coli*, we have found that several 5,6-disubstituted furazano[3,4-*b*]pyrazine derivatives such as **1** and **2** have exhibited activity against Gram-positive bacteria.



1



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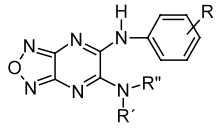
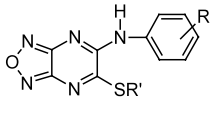


I



II

In order to study the antibacterial properties shown by this family of compounds and to improve physicochemical properties such as its low solubility in water, we started a systematic chemical program aimed at the preparation of both symmetrical and asymmetrical 5,6-disubstituted derivatives. Thus, compounds of general formula **I** and **II** became of great interest to us.

Polychloride-substituted nitrogen heterocycles such as 2,4,6-trichloro-1,3,5-triazine allows for the stepwise nucleophilic substitution of the chlorine atoms in solution leading to heterocycles bearing different residues at every position substituted.[4] However, in our case substitution of the first chlorine does not inactivate the substrate enough to prevent the second substitution from occurring. For this reason, asymmetric substitution in solution was a difficult task because it led to complex mixtures of symmetrical and asymmetrical products which needed careful chromatographic purifications. In this way, solution synthesis of compounds **I** and **II** (Scheme 1) was tackled by reaction between dichloroderivative **3** and 2 equiv. of 3-trifluoromethylaniline (1 equiv. as hydrogen chloride acceptor), followed by the introduction of a nitrogen or sulphur nucleophile according to a procedure previously reported.[5]

Despite the great reactivity showed by dichloroderivative **3** towards nitrogen nucleophiles, we have observed that the displacement of just one chlorine atom can be partially controlled by using weak bases such as the corresponding aniline as hydrogen chloride acceptors

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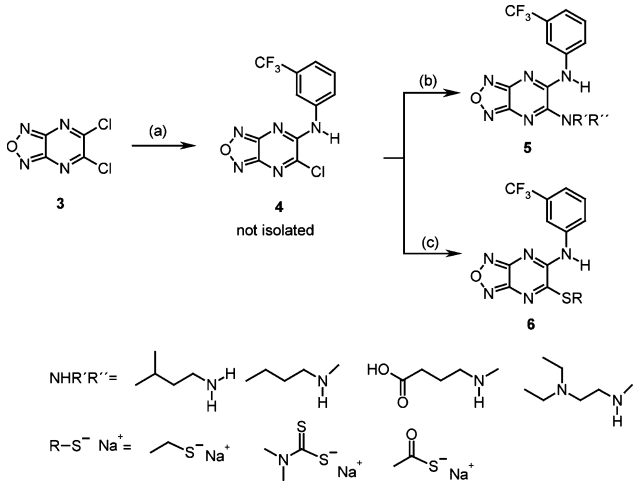
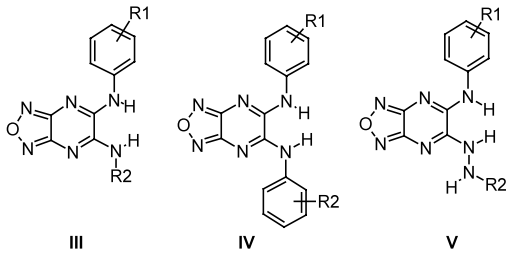


Scheme 1. *Reagents and conditions*: (a) 3-(Trifluoromethyl)-aniline (2 equiv.), acetonitrile, room temperature; (b) primary or secondary amines, acetonitrile, room temperature; (c) sodium thiolate, acetonitrile, room temperature.

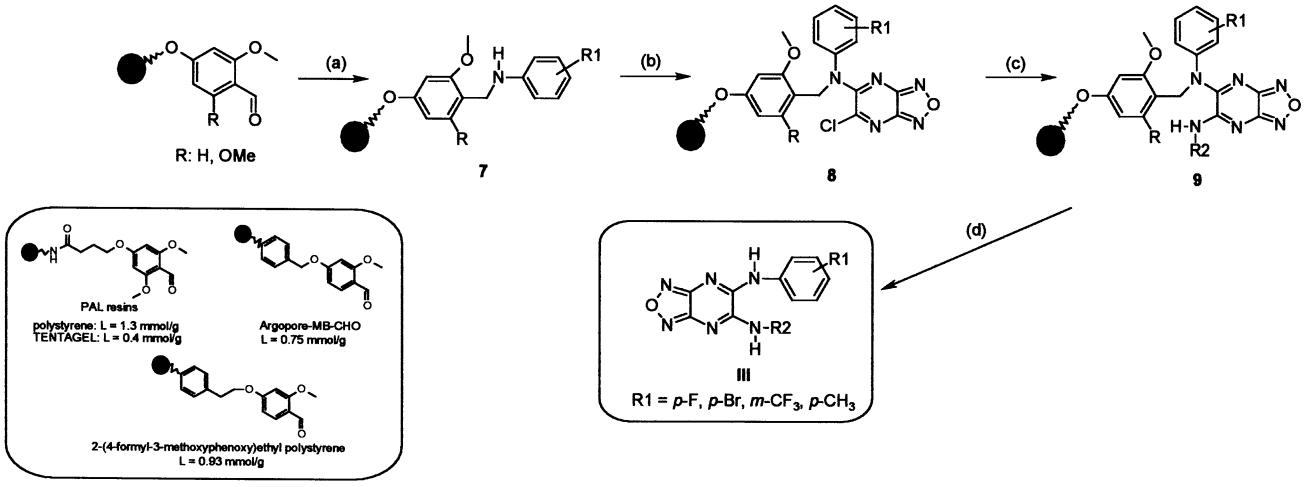
and hindered nucleophiles.[5] However, although this strategy decreases the amount of symmetrical derivatives formed, it does not avoid tedious chromatographic purifications and also narrows the scope of anilines available.

At this point, we decided to design a new strategy for the preparation of asymmetrical derivatives based on the use of solid-phase organic synthesis, which would allow us to perform the selective attachment of 5,6-dichlorofurazano[3,4-*b*]pyrazine to a solid support by displacement of one chlorine atom, leaving the second one available for an additional nucleophilic attack with a variety of nucleophiles. Hopefully, the use of solid supports not only would avoid the formation of symmetrical derivatives but would also allow us to get the final products in high purity after cleavage. Target compounds were aimed at general formulas **III**, **IV** and **V** which were of therapeutic interest within the antibacterial project.

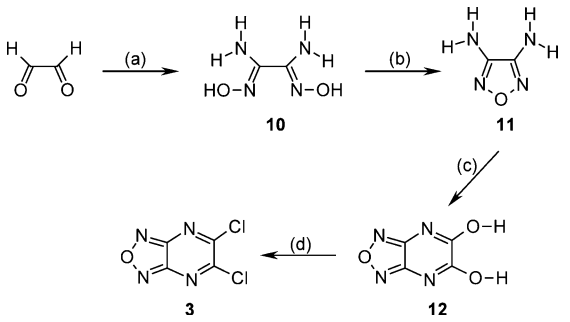


The general solid-phase synthesis of target molecules **III** is depicted in Scheme 2. The attachment of our first set of monomers, anilines, was achieved via reductive amination carried out on a di- or tri-alkoxyaldehyde loaded into a solid support, resulting in an acid labile benzyl linker.[6,7] Several conditions were tried including different solvents (DCM, DMF, DMF/TMOF), reducing agents ($Me_4N(OAc)_3BH$, $NaBH_3CN$, $Na(OAc)_3BH$), additives (AcOH, benzotriazole), reaction times and temperature. Best conditions were obtained with 2 M $Na(OAc)_3BH$ in DMF/1% acetic acid at room temperature, carrying out the reductive alkylation twice. Yield evaluation was performed by acylation of a small amount of resin **7** with acetyl chloride, cleavage with 95% trifluoroacetic acid (TFA) in dichloromethane (DCM) and ^1H NMR of the residue with 2,5-dimethylfuran (DMFu) as internal standard.[8] Yields measured in this way ranged from 55 to 100%. In general, lowest yields were obtained with those anilines bearing electron-withdrawing groups such as the *m*-trifluoromethylaniline and the ones specially hindered such as the *o*-chloro-*p*-bromoaniline.

Optimization of the introduction of dichloroderivative **3** was the following step. The highly reactive 5,6-dichlorofurazano[3,4-*b*]pyrazine **3** was prepared as a stable, tractable and crystalline solid, in multigram scale and in very good yield and high purity from glyoxal, hydroxylamine hydrochloride and oxalic acid following the synthetic route depicted in Scheme 3.[1,5]



Scheme 2. *Reagents and conditions*: (a) (i) aniline 1 M, DMF, 2% AcOH, 1 h, room temperature; (ii) $NaBH(OAc)_3$, overnight, room temperature (two couples); (b) 5,6-dichlorofurazano[3,4-*b*]pyrazine **3** (5 equiv.), DIEA, ACN, room temperature, 7 h; (c) primary amines 1 M, DIEA, ACN, room temperature, overnight; (d) TFA 25%/DCM, 3 h, room temperature.



Scheme 3. *Reagents and conditions*: (a) aq. NaOH, hydroxylamine, 5°C to reflux; (b) aq. KOH, 180°C, steel reactor; (c) oxalic acid, aq. HCl, reflux; (d) $PCl_5/POCl_3$, reflux.

Attachment of **3** to the resin was best achieved with acetonitrile as solvent at room temperature.[9] DCM and longer reaction times (overnight) were the conditions initially used but we found problems when using HiTOPS microtiter plate reaction block. HiTOPS device keeps solvent and reagents in the fritted microtiter plate wells by applying an inert gas pressure at the bottom of the plate. The nitrogen bubbling causes dryness of the resin when DCM is used as solvent. Consequently, reaction remains uncompleted and eventually moisture exposure causes some hydrolysis to produce variable amount of 6-anilino-5-hydroxyfurazano[3,4-*b*]pyrazine. To avoid this problem we finally used MeCN as solvent and 7 h treatments. Due to the high reactivity of the second chlorine, yield and purity were not determined by direct cleavage, but instead, reaction with isoamylamine (1 M) in the presence of 1 M DIEA in MeCN was carried out and cleavage with TFA in DCM done afterwards. The low basicity of the nitrogen attached to the resin made possible to reduce the amount of trifluoroacetic acid needed for cleavage to 25%.

Four different resins were evaluated (Scheme 2). Two of them bearing PAL linker (5-(4-formyl-3,5-dimethoxy-phenyloxy)valeric acid)[9] supported either in polystyrene or TentaGel™ resins, (4-formyl-3-methoxyphenoxy)methyl in Argopore resin[6] and 2-(4-formyl-3-methoxyphenoxy)ethyl polystyrene.[10] PAL linker based resins were initially used due to their milder cleavage conditions but no results were obtained when the reductive amination was carried out probably due to steric hindrance caused by the two methoxy groups vicinal to the aldehyde. With the Argopore derivatized resin, a highly-crosslinked macroporous polystyrene, desired final compounds were obtained in moderate yields but the benzyl ether spacer seemed to be sensitive to the cleavage conditions and by-products containing this residue were obtained. Best results in yields and purity were obtained with 2-(4-formyl-3-methoxyphenoxy)ethyl polystyrene which was then selected for the generation of the libraries.

Once the route of synthesis was optimized the monomer validation was performed. After reaction assessment with anilines that already showed good antibacterial activity in the furazano[3,4-*b*]pyrazine nucleus, four of them were chosen for the production of the above proposed libraries: *p*-fluoro, *p*-bromo, *m*-trifluoromethyl and *p*-methylaniline. An initial set of more than 200 amines were used to generate a virtual library using ADEPT tool[11] (A Daylight Enumeration and Profiling Tool) and it was refined by cluster analysis and predicted solvation energies as a way to improve the solubility of final compounds. After chemical rehearsal, 80 primary amines from the 116 initial set were selected including alkyl, benzyl and phenethyl amines as well as α-aminoacids and other amines with additional funtionalities. In general, a wide range of amines worked well and only the most polar ones failed to react, probably due to poor solubility in the reaction conditions. Specifically non-protected α-aminoacids, aminoalcohols and some heterocyclic amines did not work in our reaction conditions.

All final samples of compounds of general structure **III** were analyzed by LC–MS and 10% of products were weighted for yield evaluation (Table 1).[12] 97% of desired compounds were present in the library and purities depended on the aniline used: compounds bearing *p*-fluoroaniline have purities between 70 and 95%, *p*-bromo have purities between 65 and 85%, *m*-trifluoromethyl have purities above 50% (impurified with *m*-trifluoromethylaniline) and finally, *p*-methyl derivatives have purities greater than 80%. In summary, approximately 75% of compounds have purities higher than 70%. Variable amounts of *m*-trifluoromethylaniline found in the final samples might have originated because the remaining imine did not reduce in the reductive amination step.

To produce libraries of general structures **IV** and **V** we followed the same approach as in the above case (Scheme 1), but introduction of the second nucleophile needed optimization. A wide range of conditions were tried changing solvent (DCM, acetonitrile, dioxane and DMF), temperature (from room temperature to 100°C) and base (DIEA and *N*,*N*-diethylaniline). Solvents and temperature did not have a great influence improving final yields and purities. However, the use of a weaker base than DIEA gave much better results, in accordance with the results obtained in solution chemistry. Best conditions found were the use of acetonitrile as solvent at room temperature and *N*,*N*-diethylaniline as base. After monomer rehearsal 12 anilines and 17 hydrazines were chosen. In this case *m*-trifluoromethylaniline was not included in the libraries due to the moderate yields obtained in the first library. Production of both libraries was performed in the HiTOPS device as in the above library and quality control was carried out on all samples by LC–MS obtaining a medium purity of 70% for all the compounds.

In conclusion, we have developed a successful new solid-phase approach to libraries of asymmetrically disubstituted furazano[3,4-*b*]pyrazines that overcome the problems found in solution chemistry, allowing the sequential introduction of two different substituents without the formation of undesired symmetrical deriva-

Table 1. HPLC purity from selected compounds of libraries **III–IV**

Compd	R1	R2	HPLC purity[a]	MS[b]
III.1	*p*-F	1-Phenylethyl	91	351
III.2	*p*-F	1-Cyclohexyl	93	329
III.8	*p*-F	3,4-Dimethoxybenzyl	87	397
III.10	*p*-F	2,6-Difluoro-benzyl	93	373
III.14	*p*-F	Propyl	95	289
III.27	*p*-F	3-(4-Hydroxyphenyl)-1-(methoxycarbonyl)-1-ethyl	70	425
III.100	*p*-Br	Tetrahydrofuran-2-ylmethyl	75	391
III.111	*p*-Br	2-Pyridylmethyl	84	398
III.129	*p*-Br	3-Phenyl-1,2-propanediol[c]	75	444
III.258	*p*-CH_3	2-(*N*-Morpholino)ethyl	72	356
III.263	*p*-CH_3	2-(1*H*-Indol-3-yl)ethyl	89	386
III.296	*p*-CH_3	3-(Imidazol-1-yl)propyl	89	351
IV.1	*p*-F	4-Hydroxyphenyl	88	339
IV.6	*p*-F	1*H*-Indazol-5-yl	93	363
IV.10	*p*-F	4-(Methylcarbamoyl)phenyl	86	380
IV.17	*p*-Br	2-(Phenylamino)phenyl	60	474
IV.26	*p*-CH_3	4-Hydroxy-2-methylphenyl	88	349
IV.30	*p*-CH_3	4-Aminophenyl[d]	92	334
V.4	*p*-F	5-Methoxycarbonyl-4-(trifluoromethyl)pyrimidin-2-yl	82	466
V.12	*p*-F	4-(Methoxycarbonyl)phenyl	86	396
V.19	*p*-Br	Phenyl	68	398
V.20	*p*-Br	Methyl	80	336
V.39	*p*-CH_3	2-Quinoxalinyl	61	400
V.42	*p*-CH_3	4-Carboxylphenyl	73	378
V.51	*p*-CH_3	2-Pyridyl	92	335

[a] Determined at 230 nm.
[b] M+1.
[c] Obtained from the protected diol.
[d] Obtained from the Boc-protected amine.

tives and with good yields and purities. By using these solid-phase synthetic protocols, we have synthesized 320 compounds of general structure **III**, 36 **IV** and 51 **V**. Antibacterial activity was determined and will be published in due course.

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12. General procedure for the library was as follows: (a) Aldehyde resin was shaken with a solution of the corresponding aniline (1 M) in dry DMF with 1% acetic acid for 1 h at room temperature. Sodium triacetoxyborohydride (2 M) was added as solid and the suspension was shaken overnight at room temperature. Reactants were filtered and the resin was washed with MeOH (4×), water (4×), 2% AcOH in water (4×), water (4×), MeOH (4×), DCM (4×), DMF (4×) and DCM (3×). Reaction was repeated in the same conditions as above. Loading was evaluated by acetylation and cleavage with 95% TFA/DCM of a small amount of resin. Cleaved product was evaluated by ^{1}H NMR with 2,5-dimethylfurane as internal standard. (b) Aniline resin **7** was spread in a HiTops plate as a suspension in *N*-methylpyrrolidone/dichlorobenzene 1:1. Resin was dried, washed with DMF (4×) and dry acetonitrile (3×), and shaken with a solution of **3** (5 equiv.) in dry acetonitrile and diisopropylethyl-

amine (DIEA) (5 equiv.) for 7 h at room temperature. Reactants were dried and resin directly used in the next reaction. (c) Resin **8** was shaken with a solution of amine (1 M) in dry acetonitrile and diisopropylethylamine (DIEA) (1 M) overnight at room temperature. Reactants were dried and resin was washed with DCM (4×), DMF (4×), THF (4×) and DCM (4×). Resin was dried in vacuo. (d) Resin **9** was shaken with a solution of TFA 25% in dichloromethane for 3 h at room temperature. Solution was filtered and resin was washed with EtOH (1×) and DCM (1×). Filtrates were evaporated in vacuo and products dissolved in methanol (HPLC grade) to LC–MS analysis.