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Solid Phase Synthesis of Diamides as Potential Bone Resorption Inhibitors

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Abstract—Unsymmetrical diamide libraries have been prepared by a general and versatile solid phase route, using diacid templates in combination with aromatic and aliphatic amines chosen with the help of statistical experimental design. The compounds were tested as potential inhibitors of osteoclast vacuolar ATPase.

The rapidly developing field of combinatorial chemistry and, in particular, solid phase synthesis, has had a great impact on the drug discovery process of most pharmaceutical companies.[1] Instead of the time consuming method of synthesising potential drug candidates one by one, libraries of compounds can be produced either as distinct substances in an array format or as mixtures using split-and-mix techniques.

The osteoclast V-ATPase plays an essential role in bone resorption, and inhibition of this acid pump has been shown to inhibit bone resorption both in vitro and in vivo.[2] As part of a program to identify inhibitors of bone resorption, potential isosteres of **1**, a recently published compound shown to inhibit the osteoclast vacuolar ATPase (IC_{50} 49 nM),[3] were synthesised. Such inhibitors are potentially useful for the treatment of osteoporosis, i.e. the loss of bone. Diamides **2** and **3** were found to inhibit the osteoclast vacuolar acid pump in vitro with IC_{50} values of 1.5 and 4.9 μM respectively (Fig. 1).

Aiming at increasing the in vitro potency, we set out to prepare analogues in a library format of the starting points **2** and **3** with variations both in the amino functionalised side chains as well as the central diacyl unit.

Design of Building Blocks

Obvious templates for library synthesis would be **I** and **II** (Table 1). We also selected another five dicarboxylic acids (**III**–**VII**) structurally similar to calculated low energy conformations of **1**, yielding a pool of seven dicarboxylic acids.[4]

To start with, we made calculations for a number of amine and aniline structures from in-house databases. Statistical experimental designs of amine derivatives and anilines were based on molecular properties that were calculated for both two-dimensional (2D) and three-dimensional (3D) structures. The number of freely rotatable bonds and rings (some of the parameters calculated by our in-house SaSA software[5]) were used as 2D descriptors, describing conformational flexibility at the topological level. The octanol/water partition coefficients (known to be crucially important for various types of biological activities) were calculated using the method by Al Leo and implemented in the Daylight toolkit.[6] Using the Spartan package,[7] the 3D structure of the molecules was taken into account by another group of descriptors. These consisted of two subgroups basically describing spatial (molecular volume and surface, moments of inertia etc.) and electronic (dipole moment, hardness etc.) properties of the molecules, or both properties simultaneously (octanol and water sol-

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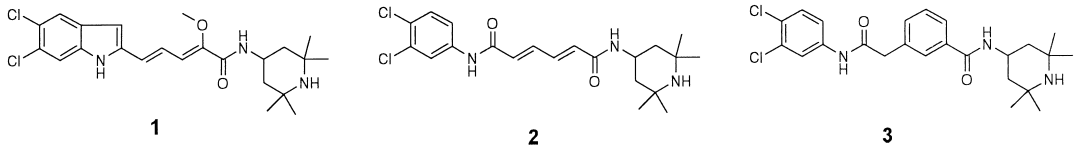


Figure 1.

Table 1. Building blocks for the diamide library

Anilines		Diacid templates	Amines	
Diverse	Similar		Aromatic	Aliphatic
A	A'	I	a	a'
B	B'	II	b	b'
C	C'	III	c	c'
D	D'	IV	d	d'
E	E'	V	e	e'
F	F'	VI	f	f'
G	G'	VII	g	g'
H	H'		h	h'
I	I'			
J	J'			

With these criteria for selection in hand, a total of 20 anilines (Table 1: **A**–**J** similar to 3,4-dichloroaniline, **A′**–**J′** as diverse as possible) and 16 amines containing additional nitrogen-based functional groups (Table 1: **a**–**h** aromatic and **a′**–**h′** aliphatic), were chosen as starting materials for the combinatorial libraries.

Chemistry

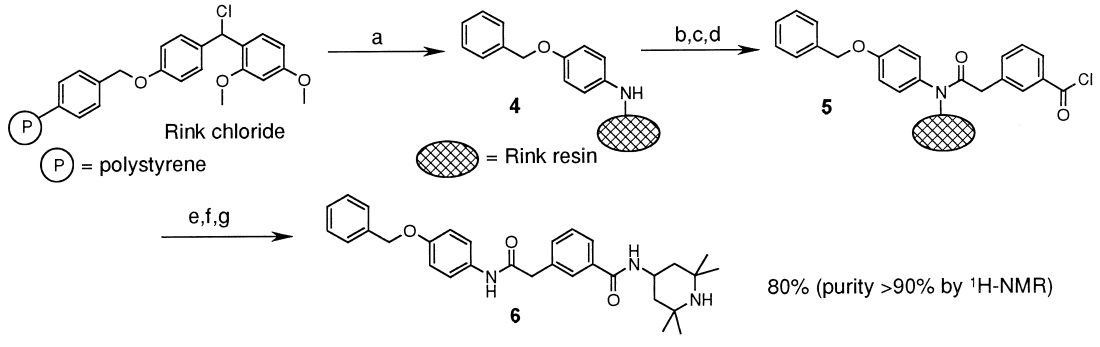
Having completed the selection of diacids and amines, we then set out to develop a solid phase route for the assembly of the desired diamide products.[8] Rink chloride,[9] prepared from Rink acid resin, has previously been used to attach anilines to a polymer backbone and thus seemed a suitable choice of linker. Furthermore, additional functionality for the resin linkage was not necessary as the amino group itself functions both as a point of attachment and a nucleophilic site. In order to avoid selectivity problems with unsymmetrical diacids, as well as to limit cross-linking and increase the solubility of the carboxylic acids, we chose to protect these as their monoesters (methyl or ethyl).[10–16] Our strategy was thus to attach the anilines to Rink chloride, couple them to our pool of diacid templates, hydrolyse the ester and then perform a second coupling step. Standard conditions for the amide bond formation (PyBOP/DIPEA or DIC/DMAP) gave incomplete reaction for most anilines, while electron poor anilines did not react at all. A more successful method was to convert the acids to their corresponding acid chloride in situ using Ghosez' reagent (1-chloro-*N,N*,2-trimethylpropenylamine).[17] The ester was subsequently hydrolysed using potassium trimethylsilanolate,[18,19] and activation of the acid that was formed was again effectuated using Ghosez' reagent. Finally, the resin bound acid chloride was reacted with an amine, and the product was then cleaved from the resin using 5% TFA in dichloromethane. Small amounts of unreacted acid were removed by filtration through a plug of silica gel. An example of a test sequence is given in Scheme 1.[20]

Once we had ascertained that synthesis of the desired diamides was possible on solid phase, we switched our attention to the preparation of diamide libraries. For practical purposes we chose to work in an 80-well format, i.e. using 10 anilines (**A**–**J** or **A′**–**J′**)×1 diacid×8 amines (**a**–**h** or **a′**–**h′**) for each plate. The selected anilines were loaded onto the Rink chloride resin batchwise and then distributed into the columns of a Flexchem solid phase synthesis block[21,22] yielding two types of starter plates; one containing anilines similar to 3,4-dichloroaniline, the other containing diverse anilines. The synthetic steps were carried out in analogy with the test reaction, using a Quadra 96 pipetting robot for addition of the reagents as well as for the washing steps. Purification was also carried out in a 96-well format, using pre-packed SPE plates with silica as the matrix. The products were subsequently weighed and analysed by MS(ES) and HPLC (254 nm).

Ten plates of compounds, based on four diacid templates (**I**–**IV**), were synthesised to start with. Approximately half of the expected 800 compounds were formed, the yields being heavily dependent on the anilines and amines used. As a general trend, electron poor anilines (similar to 3,4-dichloroaniline) gave lower yields than the diverse pool of anilines; aliphatic amines (**a′**–**h′**) gave good results while aromatic amines (**a**–**h**) performed poorly (in part due to problems with solubility). Steric hindrance in the dicarboxylic acid component was also found to be of importance; test reactions with template **VII** gave low yields even with reactive anilines and aliphatic amines. The best results were obtained when template **IV** was reacted with diverse anilines and aliphatic amines; MS analysis of this plate indicated that 80% of the desired products had been formed. An additional six compounds, based on the three remaining diacid templates (**V**–**VII**), were synthesised in parallel. The results from a representative example of a diamide library is shown in Table 2**.** In this case template **II** was reacted with diverse anilines and aliphatic amines giving the desired products in about 50% of the cases. Anilines **A**, **D**, **F** and **G** as well as amine **c′** yielded no product with template **II**.

Biology

The 10 diamide libraries were screened at 9 μM for effect on V-ATPase mediated proton transport in membrane vesicles derived from chicken medullary bone,



Scheme 1. Synthesis of diamides on solid support. Reagents and conditions: (a) 4-benzyloxyaniline (3.25 equiv), iPr_2Net (3.4 equiv), $ClCH_2CH_2Cl$, rt, 48 h; (b) acid chloride of **II** (generated in situ from **II** and Ghosez' reagent, 3 equiv), pyridine (6 equiv), CH_2Cl_2, rt, 2×2 h; (c) $KOSiMe_3$ (5 equiv), THF, rt, 3 h, then washed with 5% AcOH in THF; (d) Ghosez' reagent (4 equiv), rt, 1 h; (e) **b′** (Table 1, 5 equiv), pyridine (10 equiv), CH_2Cl_2, rt, 12+3 h; (f) 5% TFA in CH_2Cl_2, rt, 2×30 min; (g) filtration through silica.

Table 2. Yields and purities in a representative example of a diamide library[a]

		Anilines									
Diacid II		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Amines	**a′**	*x*	72 (78)	*x*	*x*	68 (86)	*x*	*x*	75 (92)	60 (80)	32 (81)
	b′	*x*	80 (81)	43 (33)	*x*	78 (84)	*x*	*x*	68 (90)	58 (64)	60 (80)
	c′	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*
	d′	*x*	69 (66)	*x*	*x*	57 (83)	*x*	*x*	71 (83)	42 (65)	27 (77)
	e′	*x*	84 (87)	51 (56)	*x*	100 (58)	*x*	*x*	75 (89)	67 (68)	32 (89)
	f′	*x*	74 (85)	*x*	*x*	60 (91)	*x*	*x*	86 (92)	53 (72)	66 (86)
	g′	*x*	79 (81)	*x*	*x*	65 (90)	*x*	*x*	71 (91)	30 (61)	67 (83)
	h′	*x*	54 (86)	*x*	*x*	59 (93)	*x*	*x*	82 (89)	52 (90)	48 (79)

[a]Purities (in parentheses) determined by HPLC at 254 nm; *x* = desired product not found by MS(ES).

using pH dependent quenching of the fluorescent weak base acridine orange (AO).[23,24] None of the compounds showed greater than 50% inhibition of the control rate of proton transport, thus no IC_{50} values were measured. In the same experiment, bafilomycin A_1 (30 nM), a specific V–ATPase inhibitor, completely abolished proton transport. The most potent compound in the diamide libraries was one of the starting compounds (compound **2**, prepared in one of the libraries as well) which gave 51% inhibition.

Discussion and Conclusions

In summary, we have shown that unsymmetrical diamides can be conveniently prepared using solid phase methods. Attachment of aromatic amines to Rink chloride provides an opportunity for linking without necessitating any extra functionality; a method that can be generalised for linking amine derivatives to solid phase. Activation of carboxylic acids towards reaction with amines and anilines using Ghosez' reagent works smoothly even on solid phase, thus avoiding side products leading to purification problems. Using prepacked SPE plates was found to be a convenient way to purify products in a 96-well format.

Statistical experimental design has proven to be a useful tool when choosing building blocks, thus reducing the need to make a large number of compounds, while still keeping the information content of diverse/similar compounds as high as possible. Due to the moderate potency of the library compounds, no clear SAR data could be obtained.

The screening of the libraries showed that there was no compound with better in vitro potency than the starting point, i.e. diamide **2**, suggesting that further production of diamide libraries would not be worthwhile.

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11. Hydrolysis and esterification of 3-cyanobenzyl nitrile, prepared from 3-cyanobenzyl bromide and acetone cyanohydrin (Dowd, P.; Wilk, K. B.; Wlostowski, M. *Synth. Commun*. **1993**, *23*, 2323) yielded **II.**
12. Compound **III** was used as purchased (Aldrich).
13. Selective hydrolysis of dimethyl *trans*-1,2-cyclopropanedicarboxylate with KOH in wet MeOH gave **IV**.
14. Compound **V** was synthesised from ethyl-2-bromo-propionoate and glyoxylic acid by use of a Wittig reaction (Zumbrunn, A.; Uebelhart, P.; Eugster, C. H. *Helv. Chim. Acta* **1985**, *68*, 1519).
15. Pyridine-2,5-dicarboxylic acid was selectively esterified giving **VI** (Isagawa, K.; Kawai, M.; Fushizaki, Y. *Nippon Kagaku Zasshi* **1967**, *88*, 553; CA 68840h 1968).

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19. Conjugate addition of $KOSiMe_3$ to the double bond was a side reaction for template **III**, but could be avoided if the reaction time was limited to 3 h.
20. General procedure exemplified by the synthesis of **6**. To a stirred solution of **II** (43 mg, 0.225 mmol) in dry CH_2Cl_2 (1.5 mL) was added 30 mg (0.225 mmol) 1-chloro-*N,N*,2-trimethylpropenylamine (Ghosez' reagent). The resulting solution was stirred for 1 h at room temperature. To a 3 mL polypropylene tube fitted with a frit containing **4** (50 mg, 0.025 mmol) swollen in CH_2Cl_2, was added pyridine (12.2 μL, 0.15 mmol, 6 equiv in 0.5 mL CH_2Cl_2) followed by 0.5 mL (0.075 mmol, 3 equiv) of the acid chloride solution above. After 2 h with occasional mixing, the solvent was removed and the resin washed with CH_2Cl_2 (3×1 mL). The procedure above was repeated. After removal of the solvent the resin was washed with CH_2Cl_2, CH_2Cl_2:MeOH 1:1, MeOH and THF. $KOSiMe_3$ (16 mg, 0.125 mmol, 5 equiv) in dry THF was added and the mixture was left for 3 h with occasional mixing. The solvent was removed and the resin washed with 5% AcOH in THF followed by THF, MeOH, CH_2Cl_2:MeOH 1:1 and CH_2Cl_2. Ghosez reagent (17 mg, 0.125 mmol, 5 equiv) in dry CH_2Cl_2 (1 mL) was added. After 1 h at room temperature with occasional mixing, the solvent was removed and the resin washed with CH_2Cl_2 followed by THF and CH_2Cl_2. Pyridine (12.2 μL, 0.15 mmol, 6 equiv) in CH_2Cl_2 (0.5 mL) was added followed by amine **b′** (12 mg, 0.075 mmol, 3 equiv) in CH_2Cl_2 (0.5 mL). The mixture was left over night. After removal of the solvent followed by washing with CH_2Cl_2, the procedure was repeated. The solvent was removed after 3 h at room temperature and the resin washed with CH_2Cl_2 followed by THF, MeOH, CH_2Cl_2:MeOH 1:1 and CH_2Cl_2. Cleavage: the resin was treated with 5% TFA in CH_2Cl_2 (2 mL) for 2 × 30 min; the filtrate and the washings were combined and concentrated. The crude material was mixed with EtOAc (0.5 mL) and added to a silica SPE column (200 mg/3mL). Elution of the product with CH_2Cl_2:MeOH 9:1 followed by evaporation of the solvents yielded **6** (10 mg, 80%). ^1H NMR (600 MHz, CD_3OD) δ 7.79 (s, 1H), δ 7.71 (d, 1H), δ 7.52 (d, 1H), δ 7.40–7.45 (m, 5H), δ 7.35 (dd, 2H), δ 7.28 (t, 1H), δ 6.93 (m, 2H), δ 5.17 (s, 2H), δ 4.47 (m, 1H), δ 3.72 (s, 2H), δ 2.14 (dd, 2H), δ 1.64 (dd, 2H), δ 1.57 (s, 6H), δ 1.46 (s, 6H). MS ES^+ 395.5, ES^- 393.5.
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24. Vesicles (20 μg protein, prepared as described[23] using calcium deprived egg-laying hens) diluted in buffer containing DTT (final concentration 0.5 mM), buffer (5 mM Hepes/Tris; pH 7.4, 3 mM $MgSO_4$, 125 mM KCl, 5 μM acridine orange, 1 μM valinomycin) and the compounds (9 μM, final concentration) or DMSO (control) were pre-incubated for 10 min at room temperature. Proton transport was then initiated by the addition of 3 mM Tris–ATP and the decrease in AO absorbance (λ = 490 nm) was followed in a 96-well plate spectrophotometer (Molecular Devices, CA, USA). The rate of proton transport was taken to be the maximum rate of decrease of AO absorbance.