Dynamic combinatorial chemistry with hydrazones: cholate-based building blocks and libraries†

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We describe an efficient and general strategy for the synthesis of dimethyl acetal functionalised steroidal hydrazides based on the cholic acid skeleton with the aim of using these compounds as building blocks for dynamic combinatorial chemistry. Deprotection of the acetal protected building blocks with TFA leads to formation of libraries containing macrocyclic N-acyl hydrazone oligomers. The isolation of several of these, and their characterisation using NMR is described. The effects on the equilibrium library distribution by varying the substituents at C-7 and C-12, extending the side-chain with glycine, and inverting the configuration at C-3 are discussed. Finally, we report the exchange properties of these macrocycles and demonstrate new examples of proof-reading and self-sorting in dynamic combinatorial libraries.

Introduction

The ability of a template molecule to direct the assembly of, or to select, its ‘ideal host’ by means of non-covalent interactions is fundamental to the success of catalytic antibodies,1 ribozymes2 and their DNA equivalents3 as well as certain imprinted polymers.4 In terms of producing catalysts and molecular hosts capable of recognition all have their attendant advantages and disadvantages.5

In the past decade, dynamic combinatorial chemistry has emerged as a powerful new templating approach for the discovery of unexpected supramolecular systems.6 In a dynamic combinatorial library (DCL), a series of receptors is allowed to interconvert in solution through reversible bond-making and bond-breaking. The reversibility gives the system the possibility to respond to the addition of a template in such a way as to reveal stabilising non-covalent intermolecular interactions. An equilibrium distribution of receptors should be perturbed by the addition of a template that binds selectively to, stabilises and hence favours the formation of one particular receptor. The DCL can be screened in situ, presenting a very attractive way of avoiding the laborious and difficult task of designing molecular hosts or guests. Elegant examples of template-directed assembly under thermodynamic control have been reported, leading to the discovery of new receptors and ligands utilising a variety of different reversible chemistries.7–9

The results reported herein build on our early work using transesterification in DCLs, where we reported a series of macrocyclic receptors based on cholate building blocks, whose equilibria can be perturbed by the addition of alkali metal ions.10

In our search for a mild alternative to transesterification, to permit the use of organic templates and foster weak non-covalent intermolecular interactions, we have been actively investigating the trifluoroacetic acid catalysed exchange behaviour of macrocyclic N-acyl hydrazones;11 the acid catalysed exchange of macrocyclic imines was reported by Wild around the same time as this work began.12 Historically, N-acyl hydrazones were for many years used for isolating and characterizing liquid aldehydes and ketones. For other applications, they have been largely neglected in the chemical literature. This is surprising, given their appealing characteristics: they are stable, easily accessible from carbonyl compounds and hydrazides, and they possess potential pseudo-peptidic recognition features (Fig. 1).13

Fig. 1  a) The reversible hydrazone reaction. b) General design of cholate-derived building blocks.

The pedigree of bile acids as supramolecular building blocks for recognition has been well established. Davis14 and
Bonar-Law and Sanders\textsuperscript{15,16} have shown that these molecules can be functionalised and cyclised to form rigid cavities, capable of recognising carbohydrates, alkaloids and metal ions, either alone, or as part of other supramolecular architectures. The purpose of the work described here was to equip a range of functionalised bile acids with a dimethyl acetal and a hydrazide and study both the range and nature of the macrocyclic N-acyl hydrazones formed upon deprotection with a view to thermodynamic templating. This work builds on our earlier studies of steroid\textsuperscript{16} and amino acid-based\textsuperscript{17} macrocyclic N-acyl hydrazones.

Results and discussion

Synthesis

Lithocholate, deoxycholate, chenodeoxycholate and cholate-based dimethyl acetal functionalised hydrazides were obtained by applying, with modifications where appropriate, the synthetic methodology shown in Scheme 1. Methyl deoxycholate (2) was synthesised, using the method of Fieser, in 85\% yield from deoxycholic acid (1).\textsuperscript{18}

Selective esterification of the diol (2) at the 3-position was achieved by means of EDC mediated coupling of 3-carboxybenzaldehyde in CH\textsubscript{2}Cl\textsubscript{2}, with a catalytic amount of DMAP, to form the ester (3) in 85\% yield.\textsuperscript{19} The aldehyde (3) was protected as its dimethyl acetal (4) by means of a catalytic amount of pTsOH in MeOH. Coupling of pyridine-4-carboxylic acid at the 12-position of 4 to form the ester (5), in 90\% yield, was also achieved by means of EDC in CH\textsubscript{2}Cl\textsubscript{2}. This required a stoichiometric amount of DMAP, however, compared to the catalytic amount sufficient for coupling at the 3-position. Efficient and selective hydrazinolysis of the methyl ester (5) to form the hydrazide (6) in 90\% yield required 30 equivalents of hydrazine monohydrate, and a concentrated solution of 5 in MeOH or EtOH at room temperature.\textsuperscript{20} The reaction typically required 48 h to reach completion. Heating was avoided, due to the presence of the aromatic esters at the 3- and 12-positions of the steroid, which react under forcing conditions.

The data in Scheme 2 summarises the pyridine-substituted steroidal hydrazides that have been prepared by the general reaction sequence depicted in Scheme 1, substituting reagents where appropriate. Thus, the synthesis of 9 required the use of pyridine-3-carboxylic acid, while 10 and 11 were synthesised from chenodeoxycholic acid (8). In order to investigate the effect of changing the geometry of a dimethyl acetal functionalised hydrazide on the range of macrocyclic N-acyl hydrazones formed at equilibrium 3-carboxybenzaldehyde was substituted for 4-carboxybenzaldehyde in the synthesis of 12.

Scheme 2  Summary of pyridine-appended building blocks. Reagents and conditions as in Scheme 1.

A second series of steroidal hydrazides, depicted in Scheme 3, does not possess pyridine-3 or 4-carboxylate substituents at the 7- or 12-positions of the steroid. The aim with this sequence of molecules was to leave free hydroxyl groups, to potentially enable recognition and binding of polyhydroxylated guests, such as carbohydrates.\textsuperscript{21} Again 4-carboxybenzaldehyde was substituted for 3-carboxybenzaldehyde in the synthesis of 17, 18 and 19. As well as being derived from deoxycholic (15 and 18), and chenodeoxycholic acid (16), one monomer (19) was synthesised from cholic acid. The lithocholate derivatives (14 and 17) were to be used as control molecules.

Scheme 3  Second series of building blocks. Reagents and conditions: See Scheme 1.

To further investigate the effect of changing the geometry and flexibility of a dimethyl acetal functionalised steroidal hydrazide on the range of macrocyclic N-acyl hydrazones formed at equilibrium the glycine extended steroidal hydrazide (21) shown in...
Scheme 4  Synthesis of extended steroid building blocks. Reagents and conditions: a) Glycine methyl ester, EDC, DMAP, Et₃N, room temperature. b) 3-Carboxybenzaldehyde, EDC, DMAP, CH₂Cl₂. c) pTsOH, MeOH. d) NH₂NH₂, MeOH, room temperature.

Scheme 4 was synthesised. The methyl ester (20) was obtained in 95% yield following EDC mediated coupling of glycine methyl ester hydrochloride with 1 in CH₂Cl₂, using a catalytic amount of DMAP and Et₃N. The alcohol (20) was selectively esterified at the 3-position with 3-carboxybenzaldehyde by means of EDC and a catalytic amount of DMAP in CH₂Cl₂. The aldehyde was protected as the dimethyl acetal by treatment with a catalytic amount of pTsOH in MeOH. Finally hydrazinolysis of the methyl ester with 20 equivalents of hydrazine monohydrate in MeOH, afforded the hydrazide (21) in 56% overall yield. The pyridine-appended extended building block (23), was prepared in 43% overall yield from the carboxylic acid (1) by the closely-related route summarized in Scheme 5.

Scheme 5  Synthesis of extended steroid building blocks. Reagents and conditions: a) Benzyl alcohol, EDC, DMAP. b) 3-Carboxybenzaldehyde, EDC, DMAP, CH₂Cl₂. c) pTsOH, MeOH. d) Pyridine-4-carboxylic acid, EDC, DMAP, CH₂Cl₂. e) Pd/C 10%, H₂. f) Glycine methyl ester, EDC, DMAP, Et₃N. g) NH₂NH₂, MeOH, room temperature.

The final variation in structure was to invert the configuration at the 3-position of deoxycholic acid (Scheme 6). This was achieved by the previously reported method by Fiegel et al. Mitsunobu coupling of formic acid with the alcohol (2) using DIAD and PPh₃ in THF led to the 3β formate ester in 85% yield.

Scheme 6  Synthesis of steroid building block with inverted stereochemistry. Reagents and conditions: a) MeOH, AcCl, room temperature. b) Formic acid, DIAD, PPh₃, THF. c) Na, MeOH, room temperature. d) 3-Carboxybenzaldehyde, EDC, DMAP, CH₂Cl₂. e) pTsOH, MeOH. f) Pyridine-carboxylic acid, EDC, DMAP, CH₂Cl₂. g) NH₂NH₂, MeOH, room temperature.

Removal of the formate group was achieved using a freshly prepared solution of sodium methoxide to afford the alcohol with the stereocentre reversed (24) in 75% yield from the carboxylic acid (1). The subsequent steps were identical to those employed in the synthesis of the hydrazide (25) leading to the hydrazide (25) in 25% overall yield from the ester (2).

Cyclisation studies

In order to favour the formation of macrocyclic N-acyl hydrazones over linear polymers, deprotection of the dimethyl acetal functionalised hydrazides was conducted under high dilution conditions. Thus, a 5 mM solution of the monomer in CH₂Cl₂, CHCl₃ or DMSO was treated with 5% TFA v/v at room temperature. The acid serves to remove the dimethyl acetal protecting group and also to catalyse formation of the macrocyclic N-acyl hydrazones. In CH₂Cl₂ and CHCl₃, deprotection of the dimethyl acetal and cyclisation to form the N-acyl hydrazones were complete in a matter of minutes, while the reaction in DMSO had to be stirred at room temperature for 24 h before all of the starting material had been consumed. For the purposes of recognition and isolation of macrocycles we were more interested in, and so focused upon, deprotection in the chlorinated solvents.

Analysis of the reaction mixtures, directly by reverse phase HPLC and ESI-MS, indicated that the cyclic N-acyl hydrazone dimers were the dominant products (>95% yield) from the deprotection of the meta substituted dimethyl acetal functionalised hydrazides (6, 9, 10, 11, 14, 15 and 16). Only trace amounts of higher macrocycles, trimer and tetramer, could be detected by ESI-MS. Increasing the concentration of the dimethyl acetal functionalised steroidal hydrazide from 5 to 20 mM did not affect
this distribution to any significant extent, suggesting that the shape of these monomers is such that they are strongly predisposed towards the formation of cyclic dimer.26

The reaction mixtures derived from the hydrazides 15 and 6 were neutralised with Et3N and subjected to preparative thin layer chromatography. The pure N-acyl hydrazone macrocyclic dimers 26 and 27 (Fig. 2 and 3) were isolated in high yields (~90%), and were soluble in acid free CHCl3, to a concentration of 20 mM.26

The 1H NMR spectrum of 26 (Fig. 2) in CDCl3, was surprisingly simple, given that one might expect conformational or geometrical isomerism about the N-acyl hydrazones. That cyclisation had indeed taken place could be readily ascertained from the disappearance of the methoxy methyl groups of 15 at 3.34 ppm, the dimethyl acetal CH singlet at 5.43 ppm, the hydrazide NH resonance, at 6.84 ppm, and hydrazide NH2 resonance, at 3.90 ppm, as well as the appearance of new signals in 26 due to the azamethine proton, Hµ at 7.55 ppm and the N-acyl hydrazone NH signal at 9.34 ppm. Other shifts, could also be observed in the aromatic and steroid regions of 26 compared with 15. Particularly noticeable was the emergence and separation of the two diastereotopic 23-H protons from the forest of steroid peaks to 2.57 and 2.96 ppm.

N-Acyl hydrazones can exist in several possible arrangements and geometrical isomers: cis E, cis Z, trans E or trans Z.27 The formation of rigid macrocycles, especially under equilibrating conditions, might be expected to favour the most stable geometry or arrangement; in this case probably an E arrangement about the N-acyl hydrazone NH. The NOESY spectrum of 26 in CDCl3, at room temperature revealed strong nOe cross-peaks between the N-acyl hydrazone resonance, at 9.34 ppm, and the azamethine proton, Hµ, at 7.55 ppm. Further nOe cross-peaks could be observed between Hµ and Hη, at 8.08 ppm. This strongly supports a cis E geometry about the N-acyl hydrazone. That the hydrazone NH, is not directed into the cavity would seem to be supported by the lack of any nOe cross-peaks between the N-acyl hydrazone NH and the steroid 22-H or 23-H resonances.

While the 1H NMR spectrum of 27 in CDCl3, was also simple, it differed from that of 26 in two noticeable respects (Fig. 3). Thus, the N-acyl hydrazone NH resonance of 27 was observed at 10.85 ppm, compared to 9.34 ppm in 26, and the azamethine proton, H0 in 27 appeared at 8.62 ppm, compared to 7.55 ppm in 26. The fact that the presence of the pyridine-4-carboxylate at the 12-position of the steroid could stabilise an otherwise disfavoured conformation was intriguing: the NOESY spectrum revealed no nOe cross-peak between the N-acyl hydrazone resonance at 10.85 ppm and the azamethine proton, H0, at 8.62 ppm, but a cross-peak was observed between H0 and Hη, at 7.87 ppm and also between the N-acyl hydrazone NH and the 23-H steroid resonances, suggesting the presence of a trans E N-acyl hydrazone geometry. A weak nOe cross-peak could also be observed between the N-acyl hydrazone NH and the α-pyridyl proton of the pyridine substituent, at 8.65 ppm. This would seem to confirm that the pyridine substituent stabilises the trans E N-acyl hydrazone conformation. The NOESY spectrum also contains exchange peaks, indicating an alternative conformation populated to a lesser extent. A CPK model of 27 suggests that a pair of intramolecular hydrogen bonds, between the pyridine substituents and N-acyl hydrazone trans NH could account for the stabilisation of this conformation, hence the downfield shift (Δδ = −1.5 ppm) of this proton, compared to the N-acyl hydrazone cis NH in 26.

In order for a template to perturb the equilibrium of a DCL, a range of host structures must be present for templates to distinguish between. DCLs containing 26 and 27 do not satisfy this requirement due to their low diversity: distribution of macrocycles was needed. A relatively simple way to change the geometry of the monomers was to switch the aldehyde from meto to para.

When a 5 mM solution of the hydrazide building block (12) in CH2Cl2 was deprotected using 5% TFA v/v at room temperature, direct analysis of the reaction mixture by HPLC and ESI-MS revealed the presence of the cyclic dimer (28, Fig. 4) and the cyclic trimer (29, Fig. 5), in a 3:1 ratio after equilibrium was reached, typically in 4–6 h. No 12 or linear, uncyclised species were observed. Increasing the concentration of 12 led to increasing amounts of trimer and above 50 mM some cyclic tetramer, as expected.28 This was an encouraging result and interesting because the overall flexibility of the molecule had not been changed, in the sense of increasing the number of rotatable bonds, merely the direction in space of the aldehyde. The cyclic dimer (28) and the cyclic trimer (29) were readily separated by preparative thin layer chromatography to afford reasonable quantities of the pure macrocycles.

The 1H NMR spectrum of the cyclic dimer (28, Fig. 4) in CDCl3, was quite different from that of its regioisomer (27, Fig. 3). As well as the usual downfield shifts of the 23-H steroid protons in 28 compared to 12, a considerable upfield shift (Δδ = −0.54 ppm) of the α-pyridyl and β-pyridyl (Δδ = −0.20 ppm) protons of 28 compared to 12 as well as the H6,ε proton was observed. This should be compared with an upfield shift of Δδ = −0.17 ppm for
the α-pyridyl protons and Δδ = 0 ppm for β-pyridyl protons of 27 compared to the hydrazide 6 and may be indicative of π–π stacking of the two pyridine substituents in 28; this is less favourable in 27, due to the meta substituted aldehyde substituent at the 3-position. The lowfield azamethine and N-acyl hydrazone resonances of 28 compared to 27, suggest the N-acyl hydrazone adopts a cis E geometry in 28.

The arrangement of the cyclic hydrazone was confirmed by the NOESY spectrum of 28 in CDCl₃ at room temperature. A strong nOe cross-peak was observed between the N-acyl hydrazone NH, at 9.17 ppm, and the azamethine proton, Hδ, at 7.64 ppm. Also an nOe cross-peak was observed between the azamethine proton, Hμ, and Hε, at 7.49 ppm. That the N-acyl hydrazone NH was not directed into the cavity was deduced from the lack of any significant nOe cross-peak between the N-acyl hydrazone NH and the 23-H steroid protons. The change of geometry from meta (27) to para (28) at the 3-position renders the trans E, N-acyl hydrazone geometry unfavourable compared to the cis E geometry. The solution phase structure was also found in the solid state. An X-ray crystal structure of 28, obtained by slow evaporation of a solution of 28 in CD₂Cl₂, revealed the geometry of the N-acyl hydrazone to be cis E.

The ¹H NMR spectrum of the macrocyclic N-acyl hydrazone trimer (29, Fig. 5) also derived from the hydrazide 12, differs from that of the cyclic dimer (28) in all of the key resonances. The azamethine proton, Hµ at 7.86 ppm and the N-acyl hydrazone NH are downfield shifted compared with 12. In this case the NH proton is extremely downfield shifted to 11.10 ppm. Also noticeable was the absence of the large upfield shift in the α and β-pyridyl protons in 29, Δδ = +0.09 ppm and Δδ = +0.05 ppm respectively, compared to 28. While the downfield shift of the N-acyl hydrazone NH suggests the presence of hydrogen-bonding, COSY and NOESY experiments on a solution of the cyclic trimer (29) in CDCl₃ support the presence of a cis E geometry. Thus, an nOe cross-peak was observed between the N-acyl hydrazone NH, at 11.10 ppm and the azamethine proton, Hµ, at 7.64 ppm. Furthermore an nOe cross-peak was observed between Hµ and Hε, at 7.70 ppm. The reason for the downfield shift of the N-acyl hydrazone NH was not clear, but it may be due to intermolecular hydrogen-bonding between two, or more, different trimer units.

If it were true that simply changing the geometry of the aldehyde at the 3-position from meta to para were responsible for increasing the stability of the cyclic trimer compared to the cyclic dimer one might expect that deprotection of the para substituted monomer 18 would result in a similar product distribution to that obtained from the deprotection of 12. This was not found, however, to be the case: deprotection of a 5 mM solution of 18 in CH₂Cl₂, led initially to a large distribution of cyclic products (dimer, trimer and tetramer), by HPLC, but over a period of several hours the amounts of trimer and tetramer decreased. At equilibrium the ratio of dimer to trimer was found to be 8:1, with no significant amounts of the tetramer. The dimer and trimer were separated by preparative chromatography, allowing ESI-MS analysis and confirmation of the HPLC assignments. The ¹H NMR spectra of the cyclic dimer and trimer could be recorded, but their poor solubility in CDCl₃ required the addition of d₄-MeOH, depriving us of the valuable structural information supplied by the N-acyl hydrazone NH signal. Increasing the concentration of 18, from 5 mM to 10 mM, led to a cyclic dimer to trimer ratio of 6:1.

The results of deprotecting the para substituted dimethyl acetal functionalised hydrazides, 17 and 19, seem to confirm that the presence of pyridine-4-carboxylate substituent at the 12-position of the steroid controls the distribution of the macrocycles formed on deprotection and, in addition, increases the solubility of the macrocycles in CDCl₃. Thus, deprotection of a 5 mM solution of 17 in CH₂Cl₂, using the standard conditions of 5% TFA v/v led to a single macrocyclic product, the dimer, by HPLC and ESI-MS. The solubility of the neutral macrocycle, derived from 17, in CDCl₃ was initially good, but after several minutes a precipitate formed, leading to broad signals in the ¹H NMR spectrum.

The N-acyl hydrazones derived from 19 were designed to afford a platform for templating experiments with polyhydroxylated molecules in CHCl₃. However, addition of TFA to a 5 mM solution of 19 in CH₂Cl₂ and CHCl₃, led to the formation of a precipitate after an hour. Filtration of the precipitate and analysis of the filtrate revealed the presence of only a single macrocycle, which does not satisfy the diversity requirements for DCLs. Carrying out the deprotection in DMSO did not lead to the formation of a
precipitate, however, still yielded only a single macrocyclic product (dimer).

In an attempt to understand the effect of flexibility on product distribution further the meta substituted glycine extended dimethyl acetal functionalised hydrazides (21 and 23) were studied. However, deprotecting 21 and 23 at a concentration of 5 mM in CH$_2$Cl$_2$ again led to the cyclic dimer as major product (>90%), with cyclic trimer making up the remainder of the product. Increasing the concentration of 21 and 23 to 20 mM did not increase the diversity of reaction products significantly.

The final modification to the steroid structure, that is, inversion at the 3-position of the steroid to form the meta substituted dimethyl acetal functionalised hydrazide (25), was examined in an attempt to increase the diversity of macrocycles at equilibrium. In the initial distribution of products identified by HPLC and ESI-MS a range of macrocyclic N-acyl hydrazones from cyclic dimer to pentamer were observed. This, however, proved to be only an ephemeral, kinetic, distribution and after 24 h the distribution of products collapsed leaving the cyclic N-acyl hydrazone dimer (30) as the only detectable product at equilibrium (Fig. 6).

![Figure 6](image1.png)

The $^1$H NMR spectrum of 30 (obtained by preparative thin layer chromatography) was, as expected, quite different from its stereoisomer (27) in that the N-acyl hydrazone NH resonance appears at 9.58 ppm in 30 and the azamethine proton at 7.79 ppm, compared with 10.85 ppm and 8.62 ppm for the same resonances in 27. The cis E geometry of 30 was confirmed by NOESY: a strong nOe cross peak was observed between the N-acyl hydrazone NH resonance at 9.58 ppm and the azamethine proton at 7.79 ppm. In addition a strong nOe cross-peak could be observed between the azamethine proton and H$_7$, at 7.50 ppm. The most dramatic difference between 27 and 30 is the large upfield shift of H$_y$ ($\Delta$H = +0.84 ppm) in 30, attributed to the close proximity of the 12-pyridine substituent in the constrained macrocycle. There does not appear to be any evidence of $\pi-\pi$ stacking between the two 12-pyridine substituents in 30.

The range of structural modifications made to the basic bile acid core has taught us about the factors that influence the distribution of macrocyclic N-acyl hydrazones at equilibrium. Thus, a meta substituted aldehyde leads to predominant formation of macrocyclic N-acyl hydrazone dimers at equilibrium, regardless of the stereochemistry at the 3-position and the presence of a glycine extension. A para substituted aldehyde on the other hand enables significant formation of macrocyclic trimer, with the caveat that a pyridine substituent must be present at the 12-position. This also has the benefit of increasing the solubility of the macrocycle in CH$_2$Cl$_2$ and CHCl$_3$. If we wish to use these macrocycles for the purposes of templating in chlorinated solvents we must therefore address these design issues and incorporate these lessons into future molecules.

**Demonstration of reversibility and exchange experiments**

In order to demonstrate that the distribution of macrocycles is at equilibrium it was necessary to show that the formation of macrocyclic steroid N-acyl hydrazones is reversible. There are two relatively simple experiments that accomplish this. The most satisfying is to take a library solution, isolate one of the macrocycles and show that this, under the reaction conditions, gives rise to the original library distribution. Indeed, when a 2.5 mM solution of pure 28 in CH$_2$Cl$_2$ was treated with 5% TFA v/v, HPLC and ESI-MS indicated the presence of both 28 and 29 in a 3:1 ratio after 8 h. When this experiment was repeated using a 1.7 mM solution of pure 29 in CH$_2$Cl$_2$ a 3:1 ratio of 28 and 29 was observed by HPLC and ESI-MS after 6 h (Fig. 7). This confirms both the reversibility of steroidal macro cyclic N-acyl hydrazone formation and that the product distribution is under thermodynamic control.

![Figure 7](image2.png)

Alternatively, reversibility can be demonstrated by an exchange experiment using two macrocycles that may be distinguished analytically. If the formation of two macrocyclic dimers is reversible they should give rise to a statistical mixture of homo and heterodimers in the presence of acid. In order to test this, a series of experiments was carried out using several dimethyl acetal functionalised hydrazides. The first experiment involved mixing the preformed macrocycles and the second experiment involved deprotecting the two dimethyl acetal functionalised hydrazide monomers in situ. These experiments were compared to see, if any, differences between kinetic and thermodynamic distributions of products.

We were particularly interested to see if the kinetic stability of macrocyclic N-acyl hydrazone dimers would prohibit ring opening and exchange, as might, perhaps, be expected for such favoured structures. If kinetic stability were the underlying cause then one might expect to see no mixing of preformed cyclic dimers.
When the monomers are deprotected in situ, however, one would expect to see the heterodimer and for this to be a stable entity. With monomers 6 and 14 the results of the two mixing experiments were surprising. No mixing of the preformed macrocyclic N-acyl hydrazones derived from 6 and 14 was observed by HPLC or ESI-MS after a period of seven days. This was taken as evidence of the kinetic stability of the dimers. When 6 and 14 were deprotected together under the same conditions, the mixed dimer could be observed by HPLC and ESI-MS, but over a period of 24 h it disappeared (Fig. 8). This surprising example of self-sorting prompted a further series of experiments using other macrocyclic N-acyl hydrazone dimers.

When equimolar 5 mM solutions of the two macrocyclic N-acyl hydrazone dimers derived from 9 and 14, were mixed at room temperature in the presence of 5% TFA v/v the formation of heterodimer could be observed by both HPLC and ESI-MS over a period of seven days. The slow nature of the reaction was, in part, to be expected due to the constrained nature of the macrocycles and the bias towards ring closure over a second ring opening. Confirmation of this result was obtained by deprotecting 9 and 14 together at the same concentration. A statistical mixture of the three possible products was observed and, significantly, did not change over a period of seven days (Fig. 9). Further experiments using the monomers 14 and 16 also indicated that exchange of macrocyclic N-acyl hydrazones is a feasible process, occurring over a period of several days. Mixing of 10 and 14 did not lead to self-sorting.

Conclusion

We have synthesised a series of steroid based building blocks for hydrazone based dynamic combinatorial chemistry containing a hydrazide and a dimethylacetal protected aldehyde. Upon treatment of a building block in organic solvents with TFA the dominant products were cyclic dimers at thermodynamic equilibrium. Larger macrocycles were present as kinetic intermediates, and macrocycles with different conformations tend to self-sort rather than mix. The use of these macrocycles for the purpose of templating in chlorinated solvents rely on careful design of the building blocks. It may be important to avoid self-templating motifs in the building blocks, however, this is an issue that can be difficult to predict.

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Notes and references


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26 The solubility of macrocyclic N-acyl hydrazones is often rather poor in chlorinated solvents. This is especially so when the solution is neutral. The addition of TFA often helps to keep the macrocycles in solution.

