Design, synthesis and evaluation of a novel series of spiroketals based on the structure of the antibacterial gyrase inhibitor novobiocin

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Molecular modelling has been used in conjunction with crystallographic and biological data in an attempt to design compounds that mimic the structure and activity of the coumarin antibiotic novobiocin 2. Calculations on four conformations of a 6,6-spiroketal system 8–11 suggest that whilst 9 should be the lowest energy, 8 should also be readily accessible and this conformation overlays well with the coumarin and sugar rings of novobiocin. Incorporation of key hydroxy and carbamate groups onto the cyclohexane and a hydrogen bond acceptor onto the aromatic ring suggest 12a as the initial target. The crystal structure of a model compound 24 shows the conformation illustrated in 9 and thus supports the molecular modelling. However, the crystal structure of novobiocin bound to a 24 kD fragment of gyrase B, the target of the coumarin antibiotics, reveals that it is the lactone carbonyl rather than the 4-oxy group which is responsible for a key interaction with an Arg residue and consequently the carboxy group in 12a is misplaced as a corresponding H-bond acceptor. Subsequently, compounds incorporating an extra aromatic (as in 13a) or heteroaromatic ring (as in 45) bearing an H-bond acceptor, along with the extra dimethyl groups on the cyclohexane ring, have been designed. Models of these structures overlay convincingly with novobiocin bound to 24 kD gyrase B. Versatile synthetic routes have been developed allowing these compounds and the underlying hypotheses to be tested. Unfortunately, none of the compounds demonstrates significant enzyme or antibacterial activity, which we attribute to a combination of features, including the lack of a replacement for the noviose methoxy group and the failure to achieve a good stacking (charge transfer) interaction with Arg-76.

Introduction

DNA Gyrase is a bacterial enzyme responsible for the process of supercoiling DNA.[1] It is an enzyme that is vital to bacteria, has no direct counterpart in mammals and consequently it represents an attractive antibacterial target. Structurally the enzyme has two discrete subunits (A and B) and it functions as an A_2B_2 tetramer in which the A subunits are responsible for the binding, breaking and recombination of the DNA and the B subunits 'power' the process by the hydrolysis of ATP.

Two discrete classes of clinically effective antibiotics are known to act at DNA gyrase.[2] These are the quinolones and the coumarins, typified by ciprofloxacin **1** and the natural product novobiocin **2** respectively. A third class of compounds currently under investigation include the natural products cyclothialidine[3] **3**, a variety of synthetic analogues[4] and the closely related GR122222X[5] **4**. Several other gyrase inhibitors have been reported.[6] The quinolones bind to the A subunits forming a stable ternary complex between DNA, enzyme and drug, which is responsible for the subsequent cell death.[1] These compounds have found very widespread use as antibiotics against a whole spectrum of bacteria. The coumarins, such as novobiocin **2**, bind to the B subunit of DNA gyrase causing inhibition of the ATPase activity.[1] Novobiocin **2** itself has found limited clinical use, mostly against Gram positive organisms, but suffers from problems of toxicity and rapidly developing resistance.[7] More potent coumarins such as coumermycin A_1[8] and clorobiocin **5**[9] have physical properties, such as poor solubility, which have limited their development. Cyclothialidine **3** and GR122222X **4** also inhibit DNA gyrase by competitive binding to the B subunit.[3,5]

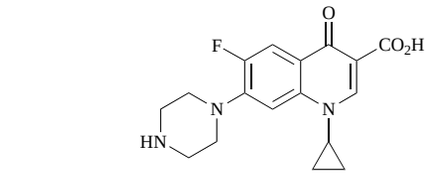
The ever increasing incidence of infection resulting from bacteria resistant to currently available therapy has prompted the continuing need to develop new and improved antibacterial agents. The inhibition of DNA gyrase, in this case specifically at the B subunit, was seen as an attractive approach.

In the past, there has been considerable work both within Zeneca[10] and elsewhere[11] to develop an improved antibacterial agent by modification of the coumarin natural products. The chemistry was highly complex and in trying to establish structure–activity relationships there was always a concern that weak levels of activity may simply be due to contamination with small amounts of the very potent parent compound. However, the structural information available for novobiocin **2** and more recently for a complex between novobiocin and a 24 kD protein fragment derived from the B subunit of *Escherichia coli* DNA gyrase, opened up the possibility of designing novel, wholly synthetic compounds which mimic novobiocin. This paper outlines the design and synthesis of a novel series of spiroketals intended to mimic those structural features of novobiocin which are believed to be responsible for binding and inactivation of gyrase B.

Results and discussion

Design

At the outset of this project the crystal structure of novobiocin **2** was available[12] (see Fig. 1). A 2D ^{1}H NMR experiment confirmed that the key conformational features seen in the solid state were also evident in solution.[13] There was also some understanding of the structure–activity relationship within the coumarins which was relevant to the design phase. First and foremost, there are features of both the aromatic portion and the sugar which seem essential for activity. Compounds having trivial replacements for either portion are inactive.[10] For the aromatic part of the molecule, it appears that replacing the terminal phenolic amide with simple amides such as isobutyroyl (as in **6**) results in lower, but still significant, activity.[10,11] Similarly, the presence of the aromatic methyl substituent (or chloro substituent in the case of clorobiocin **5**), is believed to enhance activity, but is not essential.[11] It has been shown for several bacteria, that resistance to novobiocin **2** results from a variety



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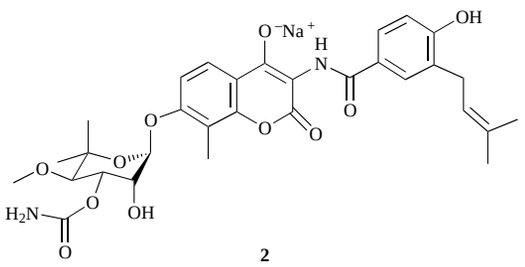

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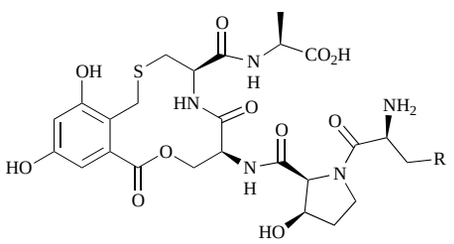
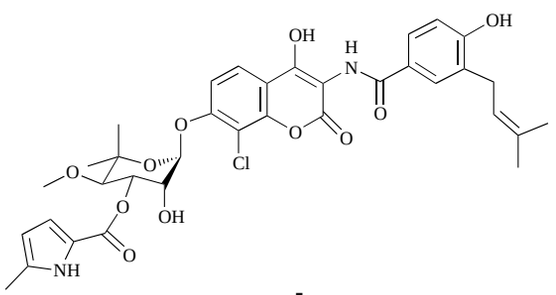


3 R = OH
4 R = H



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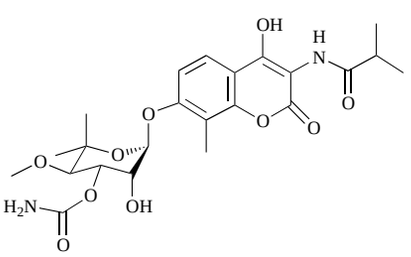


6

of point mutations of a 'key' arginine residue (equivalent to Arg 136 in *E. coli*).[14] Therefore, the interaction with Arg 136 is important for novobiocin **2** and we assumed that the 4-oxy group on the coumarin (as its anion)[15] was the most likely group responsible for this interaction. With regard to the sugar, apart from variations to the carbamate group, the limited changes that have been investigated were detrimental.[10]

We set out to design compounds containing a cyclohexyl mimic of the noviose sugar that would carry similar functionality to the natural product and place an aromatic ring in the same vicinity as the hydroxycoumarin. The aromatic ring would bear a hydrogen bond acceptor to pick up the 'key' interaction with Arg 136. There is an inherent weakness in targeting a residue which is known to be capable of mutation. However, the extent to which novobiocin is compromised in the clinic as a result of gyrase mutation is dependent on several factors which include potency and pharmacokinetics as well as the frequency and viability of the point mutation (equivalent to Arg 136 in *E.*

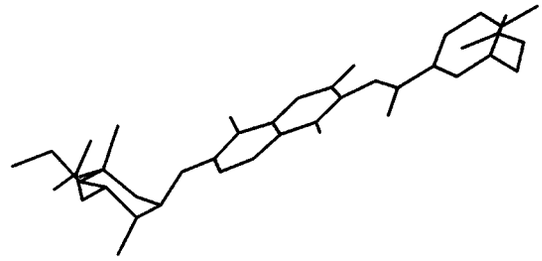


Fig. 1 Crystal structure of novobiocin

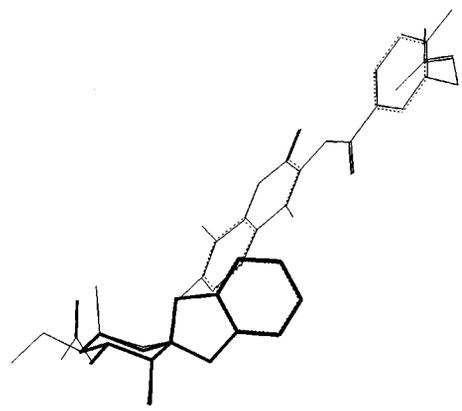


Fig. 2 Overlay of novobiocin with **7**

coli). We were optimistic that if we could identify activity in a series that had the potential for synthetic manipulation we might then be able to optimise potency and pharmacokinetics, whilst enhancing the interactions to other parts of the enzyme, thus reducing the effect of mutation of the arginine residue.

We hoped that by rigidifying the structure in an appropriate conformation we would enhance binding by reducing the entropic penalty. One problem with using a cyclohexane ring to replace the pyran of the sugar is that in the absence of the anomeric effect and without other influences the *O*-aryl substituent will occupy an equatorial rather than axial position. Consequently, we considered the possibility of having a second oxygen linked substituent in the form of a ketal to balance out this tendency. The use of a cyclic ketal to link a phenyl ring *via* two oxygen substituents to the cyclohexane ring would have the double advantage of increasing chemical stability and also freezing out the conformation. We first studied a 6,5-spiroketal in which one C–O aryl bond must inevitably be axial and the conformation would be rigidly constrained. Evaluation of a model of this structural type **7** against novobiocin **2** showed the expected overlap of the cyclohexane and pyran rings, but also revealed that the phenyl ring was not at all close in space or orientation to the phenyl ring of the coumarin (Fig. 2). We next investigated the possibility that a 6,6-spiroketal would have the correct orientation. For this system we considered four 'low energy' conformations (**8**–**11**) which represent the two ring flipped chair conformations in combination with the aromatic ring either bent towards or away from the oxygenated side of the cyclohexane ring. The relative energies of these conformations were obtained from an MM2 derived molecular mechanics potential[16] and confirmed by low level semiempirical calculations. From these calculations we immediately discount the likelihood of the conformation given in **11**. This is because even given the inherent limitations of the potential function, and an absence of a solvation model, we compute a high relative conformational energy for **11** (also confirmed by the semiempirical calculations). The relative conformational energies of the other structures (**8**–**10**) differ only by 2.1 kJ mol^{-1}. In this case the limitations of the computational model do not lead to precise conclusions about the conformational populations. However, we were satisfied that **8** is an acceptable conformation for the 6,6-spiroketal.

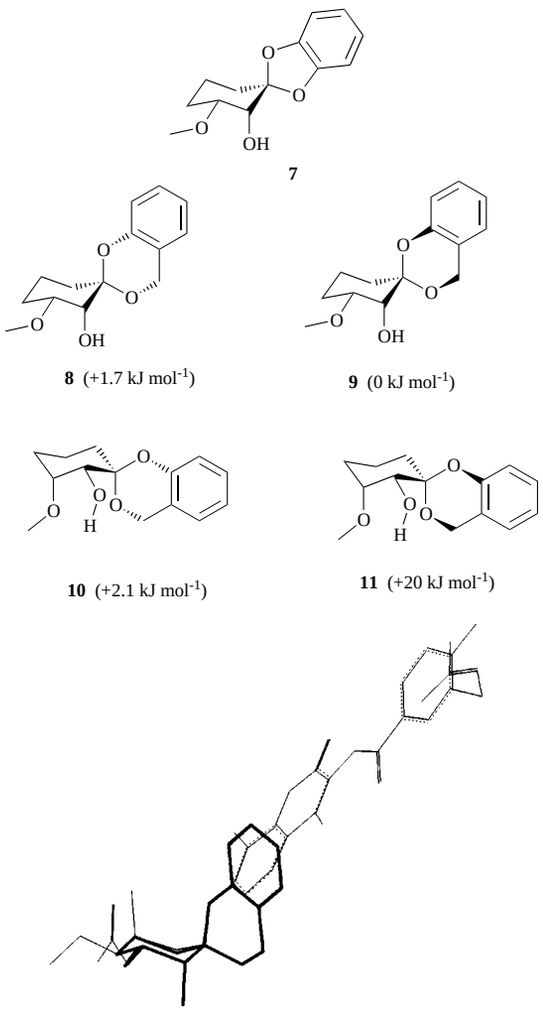
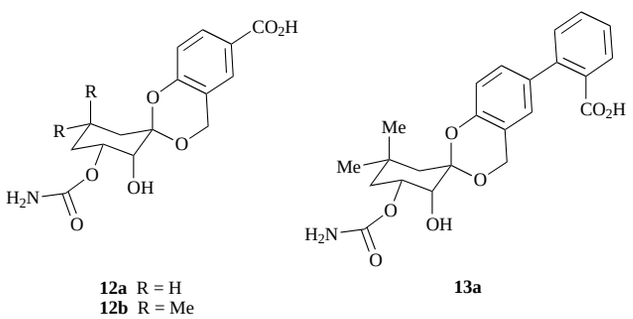


Fig. 3 Overlay of novobiocin with **8**

Comparison of **8** with novobiocin **2** showed a good overlap of both the cyclohexane and pyran rings and also the aromatic and coumarin rings (Fig. 3). The only difference observed was a slight tilt of the aromatic rings away from co-planarity. Based on these modelling results our initial target became the spiroketal **12a**, bearing oxyen functionality on the cyclohexane



ring and a carboxy group on the phenyl ring to mimic the 4-oxy group on the coumarin.[15] It was then our intention to extend our studies to amides and other hydrogen bond acceptors and also to include the *gem*-dimethyl group (as in **12b**).

During the course of this research further highly relevant structural information became available, namely, the X-ray crystal structure of a complex between novobiocin **2** and a 24 kD protein fragment derived from *E. coli* gyrase B.[17] The conformation for clorobiocin **5** in the protein–ligand complex was also solved in solution by NMR and was in agreement with that observed in the solid state.[18] Not only did the crystal structure provide information on the conformation of novobiocin **2** in a 'biologically relevant' environment, but it also clarified the

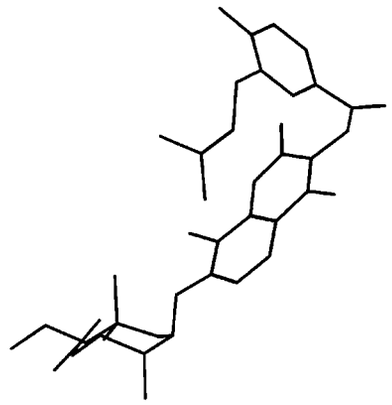


Fig. 4 Conformation of novobiocin in novobiocin–gyrase B 24 kD complex

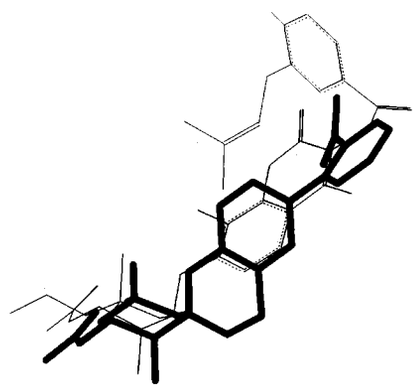


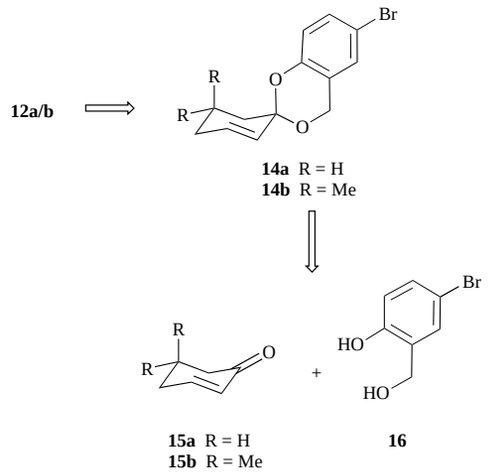
Fig. 5 Overlay of novobiocin from novobiocin–gyrase B 24 kD complex with **13a**

nature of the interactions of novobiocin **2** with those parts of the active site contained within the protein fragment. On the whole comparison of the two structures shows the conformations to be quite similar (Fig. 1 and Fig. 4), but the terminal phenolic group of the bound novobiocin appears to be bent back on itself and there is a slightly increased twist of the coumarin ring relative to the sugar. However, the protein crystal structure did reveal that it is the lactone carbonyl (as its oxyanion), rather than the 4-oxy group, that binds to Arg-136. This information implied that the carboxy group of **12a** would *not* be in the correct position to pick up the key interactions with Arg-136. As indicated earlier, the coumarin rings of novobiocin and the phenyl ring of **12a** were not strictly coplanar in our modelling. Hence, we did not believe that simply annealing a second aromatic ring would enable the correct positioning of a carboxy group. We considered whether the natural twist imparted by a biphenyl derivative would allow a second aromatic ring to be orientated in a similar manner to the coumarin ring and consequently position a hydrogen bond acceptor correctly.

Modelling the biphenyl acid **13a** in comparison to novobiocin bound to 24 kD gyrase showed that the natural twist imparted by the biphenyl put the terminal aromatic ring back into co-planarity with the coumarin and placed the carboxy group in a very favourable position to pick up the interaction with Arg-136 (as in Fig. 5). As a result of this modelling and the lack of activity seen in the early compounds our synthetic targets became the acid **13a** and related compounds. We were also interested in preparing other biaryl systems bearing hydrogen bond acceptor groups.

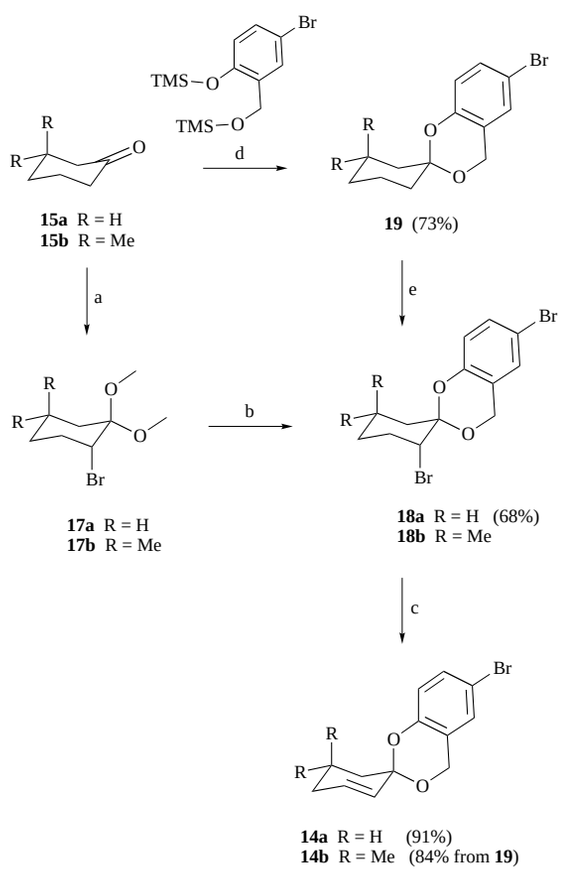
Synthesis

Our retrosynthetic analysis of the original target **12a**/**b** is illustrated in Scheme 1. It was recognised that the final products prepared by this route would be racemic but during the phase



Scheme 1

of lead identification this was deemed to be acceptable. We envisaged that **12a**/**b** could be prepared from the unsaturated compound **14a**/**b** by conversion of the double bond to the *cis*-diol and subsequent manipulation to the carbamate, along with conversion of the bromo group to a carboxylic acid. We expected that **14a**/**b** could be prepared directly from the corresponding unsaturated ketone **15a**/**b** and 5-bromo-2-hydroxybenzyl alcohol **16**. In the event we were unable to condense **15a** and **16** directly under a variety of conditions and consequently an alternative sequence was developed (Scheme 2).



Scheme 2 *Reagents:* a, MeOH, Br_2; b, **16**, PTSA, toluene; c, NaOMe, DMSO; d, TMSI, CH_2Cl_2; e, $PhMe_3N^+Br^-$

Cyclohexanone was converted directly to the bromodimethyl ketal **17a** using bromine in methanol according to the method of Garbisch.[19] Transketalisation with **16** gave the bromo spiroketal **18a** which was treated with sodium methoxide in

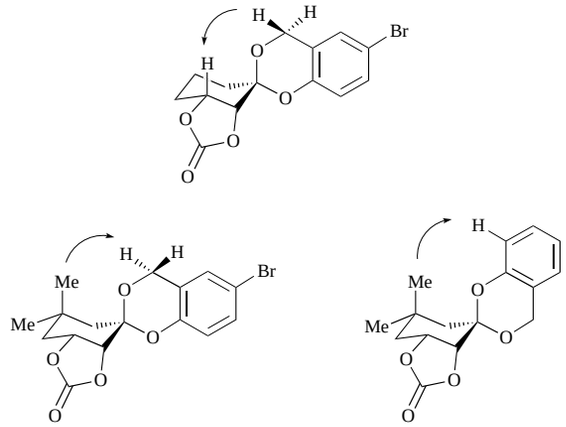


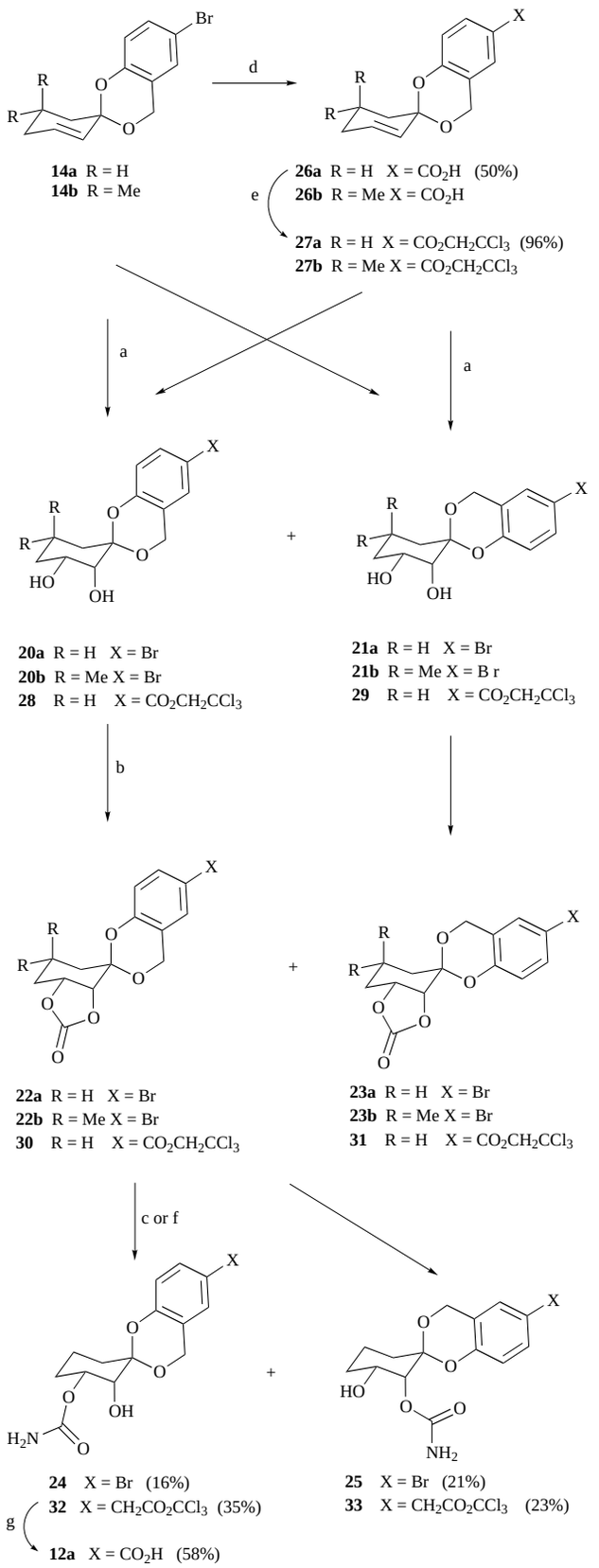
Fig. 6 Informative ^{1}H NMR NOEs

DMSO to give **14a** in 62% yield over three steps. In order to establish the relative stereochemistry and to provide structural information, the bromo derivatives themselves were progressed to final products (Scheme 3).

Dihydroxylation of **14a** gave a 1:1 mixture of the diastereomeric diols **20a** and **21a** in 54% yield, which could be separated with difficulty by HPLC. Alternatively the mixture was converted to the carbonates **22a** and **23a** with triphosgene in 81% yield,[20] which could be easily separated by flash chromatography. (Hydrolysis of the single diastereomers **22a** and **23a** gave the corresponding diols **20a** and **21a**.) Assigning the relative stereochemistry between these diastereomeric series by ^{1}H NMR spectroscopy proved to be difficult and not entirely convincing. For the des-methyl series, where the relevant peaks could be distinguished in both diastereomers (*e.g.* **22a** and **23a**), the assignment of relative stereochemistry was tentatively based on the presence of an NOE from a benzylic proton to the proton at position 3′ on the cyclohexane ring (for **22a**) and the absence of an equivalent result with the diastereomer (**23a**) (see Fig. 6). Definitive proof of the relative stereochemistry came from a single crystal X-ray structure of diol **21a**.[21]

In order to complete the synthesis of the model compound **24**, the required carbonate **22a** was reacted with ammonia to form a 1:1 mixture of the 2- and 3-carbamates **24** and **25**. In this case the two isomers were separated by HPLC with difficulty and we were able to get an X-ray crystal structure of **24**.[21] Undoubtedly it would be possible to develop a sequence of reactions that would allow selective functionalisation of the 3-hydroxy group. However, as our purpose was to identify activity in a lead series we were happy to accept the benefits that the described sequence presented, namely, straightforward chemistry and the ability to form carbamates from a variety of amines at a late stage. Except where circumstances were particularly favourable we did not separate the 2- and 3-carbamate mixtures but tested the mixture directly for biological activity.

For the synthesis of the first genuine target **12a** the bromo derivative **14a** was transmetallated and quenched with CO_2 to form the corresponding acid **26a** in 50% yield (Scheme 3). We were concerned about the base sensitivity of the final carbamate and also the acid sensitivity of the spiroketal and therefore chose the trichloroethyl ester as a protecting group for the acid that could be removed with zinc under buffered conditions.[22] In retrospect our concerns about the acid sensitivity of the spiroketal were unnecessary. The acid **26a** was converted to **27a** in 96% yield. Subsequent dihydroxylation gave diol **28** and the corresponding diastereomer **29** in 59% yield (83% based on recovered starting material). This 1:1 mixture was taken through to the carbonate derivatives **30** and **31** in 74% yield and separated by flash chromatography. The assignment of relative stereochemistry was based on ^{1}H NMR NOE experiments along with a comparison of the spectra and NOE results seen with the bromo series. Opening up of the carbonate with



Scheme 3 *Reagents and conditions:* a, OsO_4, $O(CH_2CH_2)_2(Me)N^+O^-$ or $Me_3N^+O^-$ (54%, 1:1 **20a**:**21a**; 49%, 1:1 **20b**:**21b**, 66% based on recovered **14b**; 59%, 1:1 **28**:**29**, 83% based on recovered **27a**); b, triphosgene (81%, 1:1 **22a**:**23a** from **20a** + **21a**; 76%, 1:1 **22b**:**23b** from **20b** + **21b**; 74% 1:1 **30**:**31** from **28** + **29**); c, NH_3, EtOH; d, Bu^nLi, CO_2; e, DCCI, DMAP, $HOCH_2CCl_3$; f, NH_3, $HOCH_2CCl_3$ CAUTION!; g, Zn, THF, pH 7 phosphate buffer

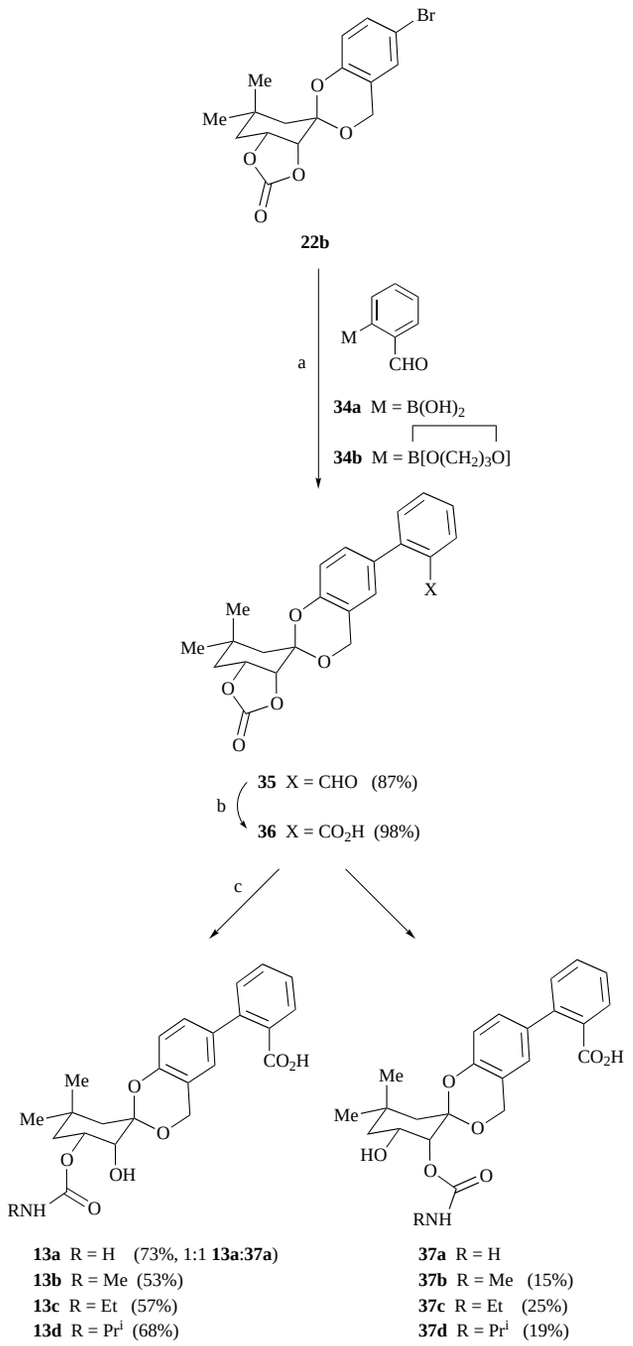
ammonia in non-alcoholic solvents gave poor results and the use of ethanol resulted in transesterification. Consequently **30** was reacted with ammonia in a small quantity of trichloroethanol (CAUTION! potential hazard) to give **32** and **33** in 58% yield, which were separated by chromatography. Deprotection with zinc in THF–phosphate buffer (pH 7) gave the target acid **12a** in 58% yield.

In tackling the synthesis of the dimethyl series (*e.g.* **13a**) we attempted to prepare the key unsaturated spiroketal **14b** using analogous chemistry to the des-methyl series (Scheme 2). However, this proved completely unsuccessful. Formation of the bromo dimethyl ketal **17b** went very poorly and under no circumstances could **17b** be converted to the bromo spiroketal **18b** even when **17b** was freshly distilled. Consequently an alternative sequence was developed (also shown in Scheme 2).

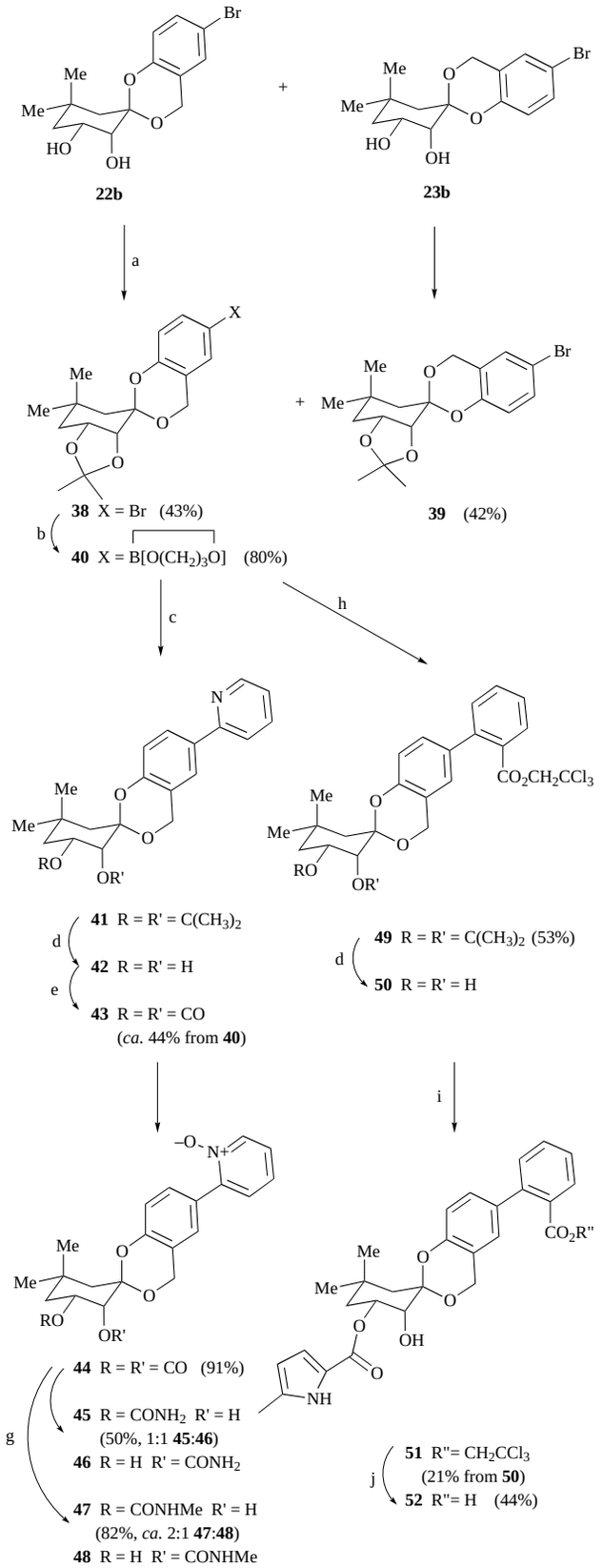
Ketalisation of **15b** with the bis (TMS) ether derived from **16** using TMSI catalysis gave **19** in good yield (73%).[23] Direct bromination could be achieved with phenyltrimethylammonium tribromide[24] and the product **18b** was converted without purification to **14b** by treatment with sodium methoxide in DMSO (84% yield). The sequence has been performed on a 1 mol scale in 40% yield over the three steps. (The lower than expected isolated yields on the larger scale are a consequence of poor recovery from the final crystallisation. No attempt was made to chromatograph the residues because **14b** proved to be fairly unstable to silica.) The unsaturated ketal **14b** was progressed through a sequence of transmetallation and quenching with CO_2 to give the acid **26b** which was protected as its trichloroethyl ester **27b**. However, this compound proved to be even less stable to silica than the bromo analogue **14b** and this instability made studies of the dihydroxylation difficult. In any event these studies were abandoned because of the change of focus towards targets such as **13a** which followed on from the protein–novobiocin structural information. However, the *cis*-hydroxylation of **14b** was still seen as a critical step and it was with some relief that it was discovered that **14b** could be converted to the diols **20b** and **21b** in 49% yield (66% based on recovered starting material) using slightly modified conditions. More importantly **20b** and **21b** and all subsequent derivatives of these compounds suffered from *no problems of stability.* As previously, the diols were converted to the carbonates **22b** and **23b** and then easily separated by flash chromatography (76% yield). In this case the relative stereochemistry could be assigned unambiguously by ^{1}H NMR NOE experiments (see Fig. 6).

It was envisaged that the biphenyl linkage in the target spiroketal biphenyl acid **13a** could be prepared by a palladium catalysed cross-coupling reaction (Scheme 4). The bromo derivative **22b** could be coupled with the *o*-formyl boronic acid **34a** using Pd^0 and CsF catalysis[25] to give **35** in 84% yield. Alternatively, **22b** could be coupled with the boronic ester **34b** using Pd^0 and Cs_2CO_3 catalysis[26] in 87% yield. The aldehyde **35** was oxidised to the acid **36** using sodium chlorite[27] in 98% yield and the carbonate group of **36** was opened with ammonia to give the target compound **13a** along with the isomer **37a** as a 1:1 mixture in 73% yield. In order to exploit potential lipophilic interactions the higher carbamates **13b–d** and their isomers **37b–d** were prepared by reaction with the corresponding amines. In these examples the ratio of isomers varied from 1:1 to 4:1 and in some cases the desired product could be isolated pure by chromatography following the preferential crystallisation of the minor isomer.

The route illustrated in Scheme 4 for the synthesis of biaryl derivatives was somewhat limiting when it came to varying the terminal aryl group because of the need to prepare boronic acids (or an equivalent organometallic derivative) for each of the aryl or heteroaryl groups in question. Consequently, we evaluated an alternative approach in which the organometallic functionality is contained within the spiroketal component and reacted with a variety of aryl or heteroaryl bromides or iodides (Scheme 5). The choice of appropriate protecting groups for the diol (**22b**/**23b**) was not an obvious one. Acyl groups would not be stable to the conditions of transmetallation and in a brief investigation we were only able to silylate the equatorial 3-



Scheme 4 *Reagents:* a, **34a**, $Pd(PPh_3)_4$, CsF or **34b**, $Pd(PPh_3)_4$, Cs_2CO_3; b, $NaOCl_2$; c, RNH_2, EtOH



Scheme 5 *Reagents:* a, $CH_3C(OCH_3)_2CH_3$, H^+; b, i, Bu^nLi, $B(OPr^i)_3$ ii, $HO(CH_2)_3OH$, toluene; c, 2-bromopyridine, $Pd(PPh_3)_4$, Cs_2CO_3; d, 1 M HCl, MeOH; e, triphosgene; f, MCPBA; g, RNH_2, EtOH; h, trichloroethyl 2-iodobenzoate, $Pd(PPh_3)_4$, Cs_2CO_3; i, 5-methyl-2-trichloroacetylpyrrole, K^-OBu^t; j, Zn, THF, pH 7 phosphate buffer

hydroxy group. We considered the dimethyl ketal because of its stability toward organometallics and suspected that it might be more labile to acid hydrolysis than the spiroketal and this turned out to be the case.

The diols **20b** and **21b** were converted to the dimethyl ketals **38** and **39** in 85% yield and separated easily by flash chromatography. The bromo group of **38** was converted by transmetallation, capture with triisopropyl borate and transesterification to the cyclic boronate ester **40** which could also be purified by chromatography (80% yield). The boronate ester **40** could be coupled with a variety of bromo- or iodo-aromatics using Pd^0 and Cs_2CO_3 catalysis[26] in high yield. For example, coupling of **40** with 2-bromopyridine gave the biaryl derivative **41** which could be cleaved very selectively under acidic conditions to give the diol **42**. This compound was converted to the carbonate **43** in an overall yield of 44% from **40**. Oxidation to the corresponding pyridine *N*-oxide **44** was accomplished using MCPBA in 91% yield and subsequent reaction of **44** with ammonia or methylamine gave the target carbamates **45** and **47** along with the corresponding isomers **46** and **48**.

The boronate ester **40** was also used to prepare a compound analogous to **13a**, but containing the 5-methylpyrrole-2-carboxylate instead of the carbamate (Scheme 5). Coupling of

40 with the trichloroethyl ester of 2-iodobenzoic acid using Pd^0 and Cs_2CO_3 as catalysts[26] gave the biphenyl derivative **49** in 53% yield. Again deprotection of the dimethyl ketal was achieved selectively and the resulting diol **50** could be selectively acylated on the 3-hydroxy group to give **51**, albeit in poor yield, using the trichloroacetylpyrrole and potassium *tert*-butoxide (21%, 27% based on recovered **50**). The trichloroethyl ester was then removed as previously using zinc in THF–buffer (pH 7) to give **52** in 44% yield.

Evaluation

The crystal structure[21] of the model compound **24** shows a conformation essentially identical to that illustrated for structure **9** and is consistent with the modelling results which predicted **9** to be the lowest energy of the four conformations modelled (**8**–**11**). Conformation **8** provides the best overlap with novobiocin (Fig. 3), but as discussed previously, we believe that the calculated energy difference between **8** and **9**, if real, is small enough to imply that **8** should still be an easily accessible conformation for our target compounds. We therefore believe that the 6,6-spiroketals do provide a scaffold on which to append both functionality equivalent to the noviose sugar and also an appropriately positioned aromatic ring, albeit with a slight twist relative to the coumarin.

All our synthetic products were tested for gyrase inhibitory activity in a standard supercoiling assay[28] at 800 μg ml^{-1} and 200 μg ml^{-1} (or 1600 μg ml^{-1} and 400 μg ml^{-1} where a 1:1 mixture of carbamates was evaluated). In this assay the coumarin standards would have the following activity: novobiocin (IC_{50} = 0.1 μg ml^{-1}), clorobiocin (IC_{50} = 0.01 μg ml^{-1}) and the semisynthetic isobutyryl derivative **6** (IC_{50} = 1 μg ml^{-1}). Very little activity was seen for any of the target compounds. The biphenyl acid **13b** showed very weak activity in the supercoiling assay (IC_{50} = 200 μg ml^{-1}), but the lack of activity in closely related compounds (**13a**,**c**,**d**) and in particular for the acyl pyrrole derivative **52**, was not encouraging. The compounds were also tested in standard antibacterial screens[29] at doses up to 128 μg ml^{-1}. The weak antibacterial activity which was seen for some of the intermediates is presumed not to be related to gyrase inhibition.

Discussion

Having seen the novobiocin–24 kD gyrase B crystal structure it is not surprising that the early compounds such as **12a** showed no activity as the carboxy group is not correctly positioned to pick up any interaction with Arg-136. These compounds also lack the *gem*-dimethyl group, which is likely to be important for binding.

There are some explanations for the lack of activity seen in the biaryl series which we do *not* believe are responsible. The most fundamental of these is that our modelling was done with a structure derived from a small fragment of the gyrase B subunit which may not be relevant to the whole enzyme. We do not believe this explanation to be the case. Whilst the 24 kD protein may not contain all the binding sites for novobiocin, microcalorimetry clearly demonstrates potent binding of novobiocin to 24 kD protein.[30] Furthermore, for selected examples of our target compounds (including **13b**) we have demonstrated by microcalorimetry that there is no binding to 24 kD protein up to the experimental limits of solubility (250 μg ml^{-1}). We have also repeated our calculations on structures analogous to **8**–**11** but bearing the *gem*-dimethyl groups and the results are very similar to those for **8**–**11**. The possibility that the oxymethylene bridge in our spiroketals is clashing with some part of the protein which is not observed in the novobiocin–24 kD structure is not supported by inspection of the 43 kD gyrB dimer structure in which the relevant section of protein is present.[31] Furthermore, if this were the fundamental cause of inactivity against the full enzyme you would still expect to see binding to 24 kD protein.

There are several factors which we believe may contribute to the lack of activity. These include the absence of the methoxy group found on the noviose sugar which may be important. Also, there appears to be a significant π-stacking (charge transfer) interaction between the hydroxycoumarin of novobiocin **2** and Arg-76, which could contribute up to 13 kJ mol^{-1} of binding energy.[32] This interaction may be significantly reduced for the target molecules as a consequence of the lack of co-planarity seen in the overlay of the aromatic ring of modelled target compounds and novobiocin. It is also possible that the terminal aromatic ring is not adequately mimicking the electronic features of the delocalised anion derived from the hydroxycoumarin. The penalty for de-solvating this delocalised anion may also be lower than that for a carboxylic acid. Compounds prepared in further studies which incorporated a double hydrogen bond acceptor in the form of a 3-hydroxypyridine *N*-Oxide as the terminal aromatic ring (and presumed to be partially ionised) also failed to show activity. In all likelihood it is a combination of features, which are really quite subtle, that result in the lack of activity and it remains a disappointment that structures that we believe have the correct conformation, carry much of the functionality believed to be important for binding and model so well into the protein, showed no activity.

Conclusions

It is an important premise of rational drug design, that different ligands that bind to the same receptor site, giving rise to a similar pharmacological response, possess a degree of molecular shape similarity. This idea has been used in the design of a series of 6,6-spiroketal derivatives based on the structure of a known inhibitor ligand (novobiocin) and its conformation bound to a 24 kD fragment of DNA gyrase. We have developed synthetic routes to these targets and the compounds appear to exhibit much of the spatial (and structural) features of the inhibitor (novobiocin). However, the compounds do not show any significant activity against DNA gyrase at concentrations of 10^4 higher than the IC_{50} of novobiocin. We believe that the lack of activity arises from a combination of features, including the lack of a replacement for the noviose methoxy group and the failure to achieve a good stacking (charge transfer) interaction with Arg-76.

Experimental

All operations were carried out at room temperature unless otherwise stated. Tetrahydrofuran (THF) was dried by distillation from calcium hydride. Flash chromatography was performed on silica (Merck Kieselgel: Art. 9385). Melting points were taken on a Buchi apparatus with the use of glass capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on Bruker WM200, WM250 or WM400 instruments and are reported as δ values (parts per million) relative to Me_4Si as internal standard. *J* Values are given in Hz. Mass spectra were recorded on VG 12-12 quadrupole, VG 70-250 SE, VG ZAB 2-SE or a VG modified AE1/Kratos MS9 spectrometers.

6-Bromospiro[4*H*-1,3-benzodioxine-2,1′-cyclohex-2′-ene] 14a

5-Bromo-2-hydroxybenzyl alcohol **16a** (13.65 g, 67.3 mmol), **17a**[19] (10 g, 44.8 mmol) and toluene-*p*-sulfonic acid (PTSA) (0.1 g) were heated at reflux in toluene, passing the condensed solvent through a Soxhlet adapter filled with 4 Å molecular sieves. After 8 h the solution was cooled and concentrated under reduced vacuum to a light brown oil which was chromatographed on silica using 10% ethyl acetate in hexane as eluent to give **18a** as a clear colourless oil which was used directly (10.95 g, 68%), δ_H(250 MHz; $CDCl_3$) 1.5–2.4 (8H, m, 4 × CH_2), 4.3 (1H, s, CHBr), 4.74 (1H, d, *J* 14, CH_AH_B), 4.94 (1H, d, *J* 14, CH_AH_B), 6.74 (1H, d, *J* 8, ArH), 7.13 (1H, d, *J* 2, ArH), 7.25

(1H, dd, *J* 3, 8, ArH); *m/z* (EI^+) 360 (M^+, 40%), 184 (C_7H_5OBr, 100).

18a (10.95 g, 30.2 mmol) was stirred in DMSO (30 ml) and NaOMe (4.77 g, 90 mmol) was added keeping the temperature below 10 °C over the addition and for the following 10 min and then allowing the reaction mixture to warm to room temperature. After 3 h the mixture was poured into brine (150 ml) and extracted with 2-methylpentane (200 ml). The organic extract was dried ($MgSO_4$), filtered and evaporated to give **14a** as a clear oil (7.8 g, 91%), δ_H(250 MHz; $CDCl_3$) 1.83 (2H, m, CH_2), 2.0 (2H, m, CH_2), 2.13 (2H, m, CH_2), 4.8 (1H, d, *J* 14, CH_AH_B), 4.9 (1H, d, *J* 14, CH_AH_B), 5.82 (1H, d, *J* 10, $CHCHCH_2$), 6.05 (1H, dt, *J* 3, 10, $CHCHCH_2$), 6.73 (1H, d, *J* 8, ArH), 7.12 (1 H, d, *J* 2, ArH), 7.26 (1 H, dd, *J* 2, 8, ArH); *m/z* (EI^+) 280 (M^+, 75%), 184 (C_7H_5OBr, 65), 96 (C_6H_8O, 100).

6-Bromo-5′5′-dimethylspiro[4*H*-1,3-benzodioxine-2,1′-cyclohex-2′-ene] 14b

5-Bromo-2-hydroxybenzyl alcohol **16a** (4.54 g, 22 mmol) was dissolved in THF (50 ml) and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (10.2 ml, 55 mmol) was added. The solution was stirred under argon at 55 °C for 30 min and then concentrated under vacuum. The residual material was diluted with 2-methylpentane and filtered through a sinter and the filtrate concentrated to give the bis-TMS ether (7.96 g, 100%).

A portion of this material (2.6 g, 7.45 mmol) and 3,3-dimethylcyclohexanone (940 mg, 7.46 mmol) were dissolved in CH_2Cl_2 (30 ml) and cooled to −78 °C under argon. Trimethylsilyl iodide (*ca.* 50 mg) was added and the reaction mixture was stirred at −78 °C for 50 min and warmed to 0 °C and stirred for a further 30 min. Saturated aqueous $NaHCO_3$ (30 ml) was added, followed by Et_2O (60 ml). The organic layer was washed with $NaHCO_3$ (sat. aq., 20 ml), $Na_2S_2O_5$ (sat. aq., 20 ml) and brine (20 ml), dried over $MgSO_4$–Na_2CO_3 (1:1), filtered and concentrated to give **19** as a colourless oil (1.69 g, 73%), δ_H(250 MHz; $CDCl_3$) 0.97 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.34 (2H, m, CH_2), 1.54–1.74 (6H, m, 3 × CH_2), 4.73 (1H, d, *J* 15, OCH_AH_B), 4.83 (1H, d, *J* 15, OCH_AH_B), 6.69 (1H, d, *J* 9, ArH), 7.08 (1H, d, *J* 2, ArH), 7.23 (1 H, dd, *J* 2, 9, ArH); *m/z* (EI^+), 310 (100%, M^+).

To a stirred solution of **19** (7.8 g, 25.16 mmol) in THF (166 ml) at 0 °C under argon was added rapidly phenyltrimethylammonium tribromide (97%; 9.75 g, 25.16 mmol). The resulting solution was stirred for 2 h at 0 °C and then diluted with $NaHCO_3$ (sat. aq., 12 ml) and stirred for a further 30 min at 0 °C. The mixture was concentrated under reduced pressure without heating to remove most of the THF and the residue was extracted with Et_2O (2 × 100 ml). The combined organic layers were washed with brine (50 ml), dried over $MgSO_4$, filtered and concentrated. The resulting oil was diluted with hexane (60 ml), filtered and the filtrate concentrated under vacuum to give **18b** as a pink gum (9.225 g, 94%) which was dissolved in DMSO (50 ml) and stirred at 0 °C under argon. NaOMe (3.85 g, 71.3 mmol) was added and the reaction mixture was stirred for 3 h at room temperature and then poured into brine (250 ml) and extracted with hexane (2 × 150 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum to give **14b** as a yellow solid (6.56 g, 89%). For analytical purposes a small sample (1 g) was recrystallised from 2-methylpentane (7 ml) to give **14b** as yellow crystals (680 mg). (*NB* **14b** is not completely stable to chromatography on silica), mp 111–114 °C (Found: C, 58.7; H, 5.6. $C_{15}H_{17}BrO_2$ requires C, 58.3; H, 5.5%); δ_H(200 MHz; $CDCl_3$) 1.02 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.87 [d, *J* 5, $CH_2C(OR)_2$], 1.97 (2H, dd, *J* 1.5, 4, $CHCH_2$), 4.85 (2H, s, OCH_2), 5.81 (1H, ddd, *J* 10, 1, 1, CH_2CHCH), 5.98 (1H, ddd, *J* 10, 3, 3, CH_2CHCH), 6.70 (1H, d, *J* 8, ArH), 7.10 (1H, d, *J* 2, ArH), 7.25 (1H, dd, *J* 8, 2, ArH); *m/z* (CI^+) 309 (MH^+, 60%), 125 ($C_8H_{13}O^+$, 100).

(2*R**,2′*R**,3′*R**)- and (2*S**,2′*R**,3′*R**)-6-Bromo-2′,3′-dihydroxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 20a and 21a

14a (2.0 g, 7.1 mmol) and *N*-methylmorpholine *N*-oxide (1.43 g, 10.6 mmol) were dissolved in Bu^{t}OH (40 ml) and OsO_4 (4% aq., 0.56 ml, 0.07 mmol) was added followed by water (1 ml). After stirring the solution for 3 days the reaction was quenched by the addition of solid sodium metabisulfite (*ca.* 300 mg) and stirring for 30 min. The mixture was evaporated to dryness under vacuum and chromatographed using ethyl acetate–hexane (1:1) as eluent to give a mixture of **20a** and **21a** as a colourless oil (1.2 g, 54%). For synthetic purposes this mixture was used directly. For analytical purposes a sample of the mixture (246 mg) was purified by HPLC (Dynamax C18 column) using 60% methanol–40% water to give **20a** (96 mg) and **21a** (71 mg) as white solids. **20a**, mp 114–116 °C (Found: C, 49.4; H, 4.6. $C_{13}H_{15}BrO_4$ requires C, 49.5; H, 4.8%); δ_H(400 MHz; $CDCl_3$) 1.4–1.9 (6H, m, 3 × CH_2), 2.32 (1H, d, *J* 8, CH_2CHOH), 2.54 (1H, d, *J* 3, $CH_2CHOHCHOH$), 3.92 (1H, m, $CH_2CHOHCHOH$), 3.97 (1H, br m, $CH_2CHOHCHOH$), 4.87 (2H, s, OCH_2), 6.74 (1H, d, *J* 8, ArH), 7.10 (1H, d, *J* 2, ArH), 7.27 (1H, dd, *J* 2, 8, ArH); δ_C(100 MHz; $CDCl_3$) 17.76 ($CH_2CH_2CH_2$), 27.34 [$CH_2C(OR)_2$], 28.46 (CH_2CHOH), 59.88 (OCH_2), 69.59 (CH_2CHOH), 71.83 ($CH_2CHOHCHOH$), 101.05 [$C(OR)_2$], 113.05 (CBr), 118.95 ($CHCHCBr$), 121.51 (CCH_2O), 127.47 (CHCBr), 131.28 (CHCBr), 149.36 ($COCR_2O$); *m/z* (CI^+) 314 (M^+, 50%), 297 ($M^+ - OH$, 50), 185 (C_7H_6OBr, 100). **21a**, mp 160–161 °C (Found: C, 49.8; H, 4.8. $C_{13}H_{15}BrO_4$ requires C, 49.5; H, 4.8%); δ_H(400 MHz; $CDCl_3$) 1.35–1.9 (6H, m, 3 × CH_2), 2.28 (1H, br d, *J* 8, CH_2CHOH), 2.49 (1H, s, $CH_2CHOHCHOH$), 3.9 (2H, br s, $CH_2CHOHCHOH$), 4.76 (1H, d, *J* 14, OCH_AH_B), 4.83 (1H, d, *J* 14, OCH_AH_B), 6.79 (1H, d, *J* 8, ArH), 7.10 (1H, d, *J* 2, ArH), 7.29 (1H, dd, *J* 2, 8 ArH); δ_C(100 MHz; $CDCl_3$) 17.68 ($CH_2CH_2CH_2$), 26.33 [$CH_2C(OR)_2$], 28.44 (CH_2CHOH), 59.62 (OCH_2), 69.40 (CH_2CHOH), 72.77 ($CH_2CHOHCHOH$), 101.38 [$C(OR)_2$], 113.11 (CBr), 118.93 ($CHCHCBr$), 121.56 (CCH_2O), 127.53 (CHCBr), 131.28 (CHCBr), 149.36 ($COCR_2O$); *m/z* (CI^+) 314 (M^+, 45%), 297 ($M^+ - OH$, 50), 185 (C_7H_6OBr, 100).

(2*R**,2′*R**,3′*R**)- and (2*S**,2′*R**,3′*R**)-6-Bromo-2′,3′-dihydroxy-5′,5′-dimethylspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 20b and 21b

These compounds were prepared from **14b** in an approximately 1:1 ratio [49% (66% based on recovered **14b**)] in a similar manner as described for **20a** and **21a**, with the exception that trimethylamine *N*-oxide was used as co-oxidant. For synthetic purposes this mixture was used directly. For analytical purposes careful chromatography on silica using EtOAc–hexane (2:3) gave samples of **20b** and **21b** as the predominant isomers which were crystallised from EtOAc–hexane to give pure samples. **20b**, mp 138–139 °C (Found: C, 52.4; H, 5.7. $C_{15}H_{19}O_4Br$ requires C, 52.5; H, 5.6%); δ_H(400 MHz; $CDCl_3$) 0.97 (3H, s, CH_{3ax}), 0.99 (3H, s, CH_{3eq}), 1.54 (1H, dd, *J* 5, 13, $CH_{eq}H_{ax}CHOH$), 1.605 [1H, d, *J* 14, $CH_{eq}H_{ax}C(OR)_2$], 1.635 (1H, d, *J* 13, $CH_{eq}H_{ax}CHOH$), 1.70 [1H, d, *J* 14, $CH_{eq}H_{ax}C(OR)_2$], 2.08 (1H, d, *J* 9, CH_2CHOH), 2.46 [1H, s, $CHOHC(OR)_2$], 3.91 [1H, s, $CHOH(OR)_2$], 4.14 (1H, m, CH_2CHOH), 4.82 (1H, d, *J* 15, OCH_AH_B), 4.88 (1H, d, *J* 15, OCH_AH_B), 6.70 (1H, d, *J* 8, ArH), 7.11 (1H, d, *J* 2, ArH), 7.27 (1H, dd, *J* 2, 8, ArH); *m/z* (CI^+) 360 ($M + NH_4^+$, 100%), 342 ($M + H^+$, 20). **21b**, mp 148–149 °C (Found: C, 52.1; H, 5.7. $C_{15}H_{19}O_4Br$ requires C, 52.5; H, 5.6%); δ_H(400 MHz; $CDCl_3$) 0.98 (3H, s, CH_{3eq}), 1.09 (3H, s, CH_{3ax}), 1.53 (1H, dd, *J* 5, 13, $CH_{eq}H_{ax}CHOH$), 1.64 [1H, d, *J* 14, $CH_{eq}H_{ax}C(OR)_2$], 1.67 (1H, d, *J* 13, $CH_{eq}H_{ax}CHOH$), 1.76 [1H, d, *J* 14, $CH_{eq}H_{ax}C(OR)_2$], 2.11 (1H, d, *J* 9, CH_2CHOH), 2.46 [1H, s, $CHOHC(OR)_2$], 3.92 [1H, s, $CHOH(OR)_2$], 4.03 (1H, m, CH_2CHOH), 4.74 (1H, d, *J* 17, OCH_AH_B), 4.88 (1H, d, *J* 17, OCH_AH_B), 6.75 (1H, d, *J* 8, ArH), 7.10 (1H, d, *J* 2, ArH), 7.27

(1H, dd, *J* 2, 8, ArH); *m/z* (CI$^+$) 360 (M + NH_4^+, 100%), 342 (M + H^+, 20).

(2*R,3a′*R**,7a′*R**)- and (2*S**,3a′*R**,7a′*R**)-6-Bromospiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxol]-2′-one 22a and 23a**

The mixture of **20a** and **21a** (*ca.* 1:1; 1.2 g, 3.8 mmol) was dissolved in CH_2Cl_2 (10 ml) and pyridine (1.8 g, 22.8 mmol). The solution was cooled to −78 °C under an argon atmosphere and a solution of triphosgene (564 mg, 1.9 mmol) in CH_2Cl_2 (10 ml) was added dropwise. The mixture was allowed to warm to room temperature and then quenched with ammonium chloride (sat. aq., 10 ml) and diluted with CH_2Cl_2. The organic layer was washed with 2 M HCl (10 ml), $NaHCO_3$ (sat. aq., 20 ml) and brine (20 ml). The solution was dried ($MgSO_4$), filtered and concentrated. The residue was chromatographed on silica using 30% EtOAc–hexane as eluent to give **22a** (550 mg, R_f 1:1 EtOAc–hexane 0.6) and **23a** (510 mg, R_f 1:1 EtOAc–hexane 0.4) as colourless oils which crystallised on standing (yield 81%). **22a** mp 151–153 °C (Found: C, 49.7; H, 3.7. $C_{14}H_{13}O_5Br$ requires C, 49.3; H, 3.8%); δ_H(250 MHz; $CDCl_3$) 1.65–2.15 (6H, m, 3 × CH_2), 4.70 (1H, d, *J* 6, CHOCO), 4.83 (1H, d, *J* 16, OCH_AH_B), 4.88 (1H, d, *J* 6, CHOCO), 4.95 (1H, d, *J* 16, OCH_AH_B), 6.75 (1H, d, *J* 8, ArH), 7.15 (1H, d, *J* 2, ArH), 7.3 (1H, dd, *J* 2, 8, ArH); *m/z* (Cl$^+$) 358 (M + NH_4^+, 100%). **23a**, mp 94–99 °C (Found: C, 49.3; H, 3.7. $C_{14}H_{13}O_5Br$ requires C, 49.3, H, 3.8%); δ_H(250 MHz; $CDCl_3$) 1.6–2.2 (6H, m, 3 × CH_2), 4.58 (1 H, d, *J* 6, CHOCO), 4.84 (2H, s, OCH_2), 4.92 (1H, m, CH_2CHOCO), 6.82 (1H, d, *J* 8, ArH), 7.11 (1H, d, *J* 2, ArH), 7.3 (1H, dd, *J* 2, 8, ArH); *m/z* (CI$^+$) 358 (M + NH_4^+, 100%).

(2*R,3a′*R**,7a′*R**)- and (2*S**,3a′*R**,7a′*R**)-6-Bromo-5′,5′-dimethylspiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]-benzodioxol]-2′-one 22b and 23b**

A mixture of diols **20b** and **21b** (3.83 g, 11.1 mmol) was converted to a mixture of the carbonates **22b** and **23b** using the same procedure as described above for **22a** and **23a**. The crude product started to crystallise on standing and CH_2Cl_2 (20 ml) was added and **23b** was filtered off as a white solid (1.165 g, 29%). The filtrate was concentrated under vacuum and chromatographed on silica using 10–50% EtOAc in hexane as eluent to give **22b** as an oil which crystallised on the addition of Et_2O (5 ml) and hexane (10 ml) (1.45 g, 37%) along with a further quantity of **23b** (0.4 g, 10%, total 39%). **22b**, mp 124–125 °C (Found: C, 52.1; H, 4.7. $C_{16}H_{17}BrO_5$ requires C, 52.1; H, 4.6%); δ_H(400 MHz; $CDCl_3$) 1.09 (3H, s, CH_{3ax}), 1.12 (3H, s, CH_{3eq}), 1.79–1.93 [4H, m, $CH_2C(CH_3)_2CH_2$], 4.69 (1H, dd, *J* 1.5, 7.5, $CH_2CHOCHO$), 4.84 (1H, d, *J* 16, OCH_AH_B), 4.96 (1H, d, *J* 16, OCH_AH_B), 4.98 (1H, m, $CH_2CHOCHO$), 6.71 (1H, d, *J* 8, ArH), 7.15 (1H, d, *J* 2, ArH), 7.29 (1H, dd, *J* 2, 8, ArH); irradiation of CH_{3ax} (1.09) gives an NOE enhancement of ArH (6.71); *m/z* (CI$^+$) 386 (M + NH_4^+, 100%), 368 (M + H^+, 10. **23b**, mp 178–181 °C (Found: C, 51.8; H, 4.6. $C_{16}H_{17}BrO_5$ requires C, 52.1; H, 4.6%) δ_H(400 MHz; $CDCl_3$) 1.07 (3H, s, CH_{3ax}), 1.11 (3H, s, CH_{3eq}), 1.705 (1H, d, *J* 15, $CH_{eq}H_{ax}CHO$), 1.89 [1H, dd, *J* 5.5, 14, $CH_{eq}C(OR)_2$], 1.96 (1H, d, *J* 15, $CH_{eq}H_{ax}CHO$), 1.97 [1H, d, *J* 14, $CH_{ax}H_{eq}C(OR)_2$], 4.64 (1H, d, *J* 7, $CH_2CHOCHO$), 4.83 (1H, d, *J* 16, OCH_AH_B), 4.87 (1H, d, *J* 16, OCH_AH_B), 4.98 (1H, m, $CH_2CHOCHO$), 6.79 (1H, d, *J* 8, ArH), 7.11 (1H, d, *J* 2, ArH), 7.30 (1H, dd, *J* 2, 8, ArH); irradiation of CH_{3ax} (1.07) gives an NOE enhancement of OCH_AH_B (4.85); *m/z* (CI$^+$) 386 (M + NH_4^+, 100%), 368 (M + H^+, 10).

(2*R,2′*R**,3′*R**)-6-Bromo-2′-hydroxy-3′-carbamoyloxyspiro-[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 24 and (2*S**,2′*R**,3′*R**)-6-bromocarbamoyloxy-3′-hydroxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 25**

22a (490 mg) was dissolved in sat. ethanolic ammonia (5 ml) and the solution stirred for 16 h at room temperature and then evaporated under reduced pressure to give **24** and **25** in an approximately 1:1 mixture. A sample of the mixture (400 mg) was separated by HPLC on a 5 μm silica preparative Dynamax column using 0.1% TFA–15% propan-2-ol–hexane as eluent, to give pure samples of the components as gums, along with the remainder of the material as a mixture. The gums crystallised on the addition of 30% diethyl ether–hexane (5 ml) to give white crystals of **24** (85 mg, 16%) and **25** (110 mg, 21%). **24** was recrystallised from ethyl acetate (2 ml) and hexane (10 ml) which gave single crystals of a 1:1 EtOAc adduct suitable for X-ray diffraction. **24**, mp 78–80 °C (Found: C, 48.3; H, 5.5; N, 3.2. $C_{14}H_{16}NO_5Br{\cdot}C_4H_8O_2$ requires C, 48.4; H, 5.4; N, 3.1%); δ_H(250 MHz; $CDCl_3$) 1.25 (3H, t, *J* EtOAc), 1.4–1.9 (6H, m, 3 × CH_2), 2.0 (3H, s, EtOAc), 4.08 (1H, dd, *J* 0.5, 3, C*H*OH), 4.25 (2H, q, EtOAc), 4.73 (2H, br s, NH_2), 4.83 (2H, s, OCH_2), 5.0 (1H, m, C*H*OCONH$_2$), 6.76 (1H, d, *J* 8, ArH), 7.08 (1H, d, *J* 2, ArH), 7.26 (1H, dd, *J* 2, 8, ArH); *m/z* (CI$^+$) 358 (MH$^+$, 60%), 174 ($C_7H_{12}NO_4^+$, 100). **25**, mp 140–144 °C (Found: C, 46.6; H, 4.4; N, 3.6. $C_{14}H_{16}NO_5Br$ requires C, 46.9; H, 4.5; N, 3.9%); δ_H(250 MHz; $CDCl_3$) 1.4–1.9 (6H, m, 3 × CH_2), 3.98 (1H, m, C*H*OH), 4.67 (1H, d, *J* 14, OCH_AH_B), 4.7 (2H, br s, NH_2), 4.87 (1H, d, *J* 14, OCH_AH_B), 5.12 (1H, d, *J* 3.5, C*H*OCONH$_2$), 6.67 (1H, d, *J* 8, ArH), 7.04 (1H, d, *J* 2, ArH), 7.21 (1H, dd, *J* 2, 8, ArH); *m/z* (CI$^+$) 358 (MH$^+$, 30%), 174 ($C_7H_{12}NO_4^+$, 100), 148 ($C_7H_{10}NO_3^+$, 100).

Spiro[4*H*-1,3-benzodioxine-2,1′-cyclohex-2′-ene]-6-carboxylic acid 26a

Bu^{t}Li (1.5 M in pentane; 18 ml, 27 mmol) was placed in a dry three-necked flask, fitted with a thermometer and under an atmosphere of argon. The solution was cooled in a dry ice–acetone bath to −60 °C and diethyl ether (75 ml) was added. A solution of **14a** (3 g, 10.7 mmol) in diethyl ether (3 ml) was added keeping the temperature below −60 °C. After 5 min carbon dioxide was bubbled into the solution and the reaction mixture was allowed to warm to room temperature, quenched with NH_4Cl (sat. aq., 50 ml) and partitioned. The aqueous layer was further extracted with diethyl ether (3 × 40 ml), then adjusted to pH 5 with conc. HCl and extracted with diethyl ether (40 ml) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resultant solid was slurried with 20% ethyl acetate–hexane and filtered to give **26a** as white crystals (1.31 g, 50%), mp 252–254 °C (decomp.) (Found: C, 68.3; H, 5.8. $C_{14}H_{14}O_4$ requires C, 68.3; H, 5.7%); δ_H(250 MHz; [$^2H_6$]DMSO) 1.75 (2H, m, CH_2), 1.93 (2H, m, CH_2), 2.10 (2H, m, CH_2), 4.93 (2H, s, OCH_2), 5.81 (1H, d, *J* 10, CH_2CHCH), 6.08 (1H, dt, *J* 3, 10, CH_2CHCH), 6.89 (1H, d, *J* 8, ArH), 7.72 (1H, d, *J* 2, ArH), 7.76 (1H, dd, *J* 2, 8, ArH); *m/z* (CI$^+$) 247 (MH$^+$, 20%), 97 ($C_6H_9O^+$, 100).

1,1,1-Trichloroethyl spiro[4*H*-1,3-benzodioxine-2,1′-cyclohex-2′-ene]-6-carboxylate 27a

To a stirred mixture of **26a** (1.2 g, 4.87 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (1.11 g, 5.4 mmol) and DMAP (61 mg, 0.5 mmol) in CH_2Cl_2 was added dropwise 1,1,1-trichloroethanol (800 mg, 5.35 mmol) maintaining the temperature below 5 °C with an ice bath. The mixture was stirred for 18 h at room temperature, filtered and the filtrate concentrated under vacuum to a gum which was chromatographed on silica using 20% EtOAc in hexane as eluent to give **27a** as a colourless oil which solidified on standing (1.86 g, 96%), mp 78–81 °C (Found: C, 50.9; H, 4.2. $C_{16}H_{15}C_{13}O_4$ requires C, 50.9; H, 4.0%); δ_H(200 MHz; $CDCl_3$) 1.6–2.2 (6H, m, $CH_2CH_2CH_2$), 4.95 (4H, s, $OCH_2 + CO_2CH_2$), 5.63 (1H, dt, *J* 1, 10, C*H*CHCH$_2$), 6.08 (1H, dt, *J* 3, 10, CHC*H*CH$_2$), 6.93 (1H, d, *J* 9, ArH), 7.80 (1H, d, *J* 2, ArH), 7.96 (1H, dd, *J* 2, 9, Ar); *m/z* (FAB$^+$) 377 (100%, MH$^+$).

1,1,1-Trichloroethyl (2*R,3a′*R**,7a′*R**)- and (2*S**,3a′*R**,7a′*R**)-spiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxole]-6-carboxylate 30 and 31**

These compounds were prepared from **27a** by dihydroxylation

as described for **20b**/**21b**, followed by reaction with triphosgene as described for **22a**/**23a**. The resulting oil was chromatographed on silica using 30% EtOAc–hexane as eluent to give **30** and **31** (57% from **27a**) as colourless oils and **30** crystallised on standing. **30**, Less polar, mp 180–182 °C (Found: C, 47.1; H, 3.6. $C_{17}H_{15}O_7Cl_3$ requires C, 46.7; H, 3.45%); δ_H(400 MHz; C_6D_6) 0.9–1.5 (6H, m, $CH_2CH_2CH_2$), 3.79 (2H, m, CHOCHO), 4.04 (1H, d, *J* 15, OCH_AH_B), 4.20 (1H, d, *J* 15, OCH_AH_B), 4.665 (1H, d, *J* 12, $CO_2CH_AH_B$), 4.705 (1H, d, *J* 12, $CO_2CH_AH_B$), 6.60 (1H, d, *J* 8, Ar), 7.59 (1H, d, *J* 1, Ar), 8.00 (1H, dd, *J* 1, 8, Ar); δ_C(100 MHz; $CDCl_3$), 15.30 (CH_2CH_2-CH_2CHO), 25.67 ($CH_2CH_2CH_2CHO$), 28.27 ($CH_2CH_2CH_2$-CHO), 60.31 (OCH_2), 74.35 (CO_2CH_2), 75.83 (CH_2CHO-CHO), 76.30 ($CH_2CHOCHO$), 95.09 (CCl_3), 98.60 (COROR′), 117.40 (Ar-CH), 119.20 (Ar-CCO_2), 121.86 (Ar-CCH_2), 127.55 (Ar-CH), 130.81 (Ar-CH), 154.27 (Ar-CO), 154.38 (OCO_2), 164.19 (CO_2CH_2); *m/z* (FAB^+) 459 (M + Na^+, 70%), 436 (M^+, 30), 289 (M − $OCH_2CCl_3^+$, 100). **31**, More polar (Found: M^+, 435.9851. $C_{17}H_{15}O_7Cl_3$ requires *M*, 435.9883); δ_H(400 MHz; C_6D_6) 0.8–1.7 (6H, m, $CH_2CH_2CH_2$), 3.67 (1H, d, *J* 6, $CH_2CHOCHO$), 3.81 (1H, m, $CH_2CHOCHO$), 4.11 (1H, d, *J* 15, OCH_AH_B) 4.24 (1H, d, *J* 15, OCH_AH_B), 4.67 (2H, s, CO_2CH_2), 6.77 (1H, d, *J* 8, Ar), 7.66 (1H, d, *J* 1, Ar), 7.96 (1H, dd, *J* 1, 8, Ar); δ_C(100 MHz; $CDCl_3$), 15.16 (CH_2CH_2CHO), 25.51 ($CH_2CH_2CH_2CHO$), 30.01 ($CH_2CH_2CH_2CHO$), 60.89 (OCH_2), 74.36 (CO_2CH_2), *ca.* 76 (hidden, $CH_2CHOCHO$), 78.45 ($CH_2CHOCHO$), 95.14 (CCl_3), 98.65 (COROR′), 118.10 (Ar-CH), 119.21 (Ar-CCO_2), 12.56 (Ar-CCH_2), 127.24 (Ar-CH), 130.83 (Ar-CH), 154.07 (Ar-CO), 155.00 (OCO_2) 164.34 (CO_2CH_2); *m/z* (FAB^+) 459 (M + Na^+, 80%), 436 (M^+, 50), 289 (M − $OCH_2CCl_3^-$, 100).

1,1,1-Trichloroethyl (2*R,2′*R**,3′*R**)-2′-hydroxy-3′-carbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane]-6-carboxylate 32 and 1,1,1-trichloroethyl (2*S**,2′*R**,3′*R**)-2′-carbamoyloxy-3′-hydroxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane]-6-carboxylate 33**

The cyclic carbonate **30** (200 mg, 0.45 mmol) was dissolved in 1,1,1-trichloroethanol (2 ml) and liquid ammonia (conc., 1 ml) was added with CAUTION and the reaction mixture was then stirred at room temperature overnight. The mixture was partitioned between CH_2Cl_2 (20 ml) and brine (20 ml). The organic layer washed with brine (3 × 10 ml), dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting oil was chromatographed on silica using 50–70% EtOAc–hexane as eluent to afford **32** (72 mg, 35%) and **33** (48 mg, 23%) as colourless oils. **32**, Less polar (Found: M^+, 453.0134. $C_{17}H_{18}NO_7Cl_3$ requires *M*, 453.0149); δ_H(400 MHz; C_5D_5N + CD_3CO_2D) 1.6–2.2 (6H, m, $CH_2CH_2CH_2$), 4.61 (1H, s, CHOH), 4.85 (1H, d, *J* 15, OCH_AH_B), 5.08 (1H, d, *J* 15, OCH_AH_B), 5.11 (2H, s, CO_2CH_2), 5.31 (1H, m, $CHOCONH_2$), 6.99 (1H, d, *J* 8, Ar), 7.82 (1H, d, *J* 1, Ar), 7.97 (1H, dd, *J* 1, 8, Ar); *m/z* (CI^+) 454 (MH^+, 100%). **33**, More polar (Found: M^+, 453.0131. $C_{17}H_{18}NO_7Cl_3$ requires *M*, 453.0149); δ_H(400 MHz; C_5D_5N + CD_3CO_2D), 1.57–2.04 (6H, m, $CH_2CH_2CH_2$), 4.32 (1H, m, CHOH), 4.89 (1H, d, *J* 15, OCH_AH_B), 5.18 (1H, d, *J* 11, $CO_2CH_AH_B$), 5.29 (1H, d, *J* 11, $CO_2CH_AH_B$), 5.53 (1H, d, *J* 15, OCH_AH_B), 5.85 (1H, d, *J* 1, $CHOCONH_2$), 6.99 (1H, d, *J* 8, Ar), 7.91 (1H, d, *J* 1, Ar), 8.01 (1H, dd, *J* 1, 8, Ar); *m/z* (CI^+) 454 (MH^+, 100%).

(2*R,2′*R**,3′*R**)-2′-Hydroxy-3′-carbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane]-6-carboxylic acid 12a**

The trichloroethyl ester **32** (60 mg, 0.13 mmol) was dissolved in THF (3 ml) and zinc dust (100 mg) followed by NaH_2PO_4 (1 M aq.; 0.65 ml) and H_3PO_4 (1 M aq; 0.65 ml). The mixture was stirred for 18 h and then a further portion of zinc dust (100 mg) was added. The mixture was filtered through Celite, diluted with water (10 ml) and extracted with EtOAc (10 ml). The aqueous layer was adjusted to pH 4 with conc. HCl and extracted with EtOAc (3 × 10 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give **12a** as a white solid (32 mg, 58%), mp 203–205 °C (Found: M^+, 323.0983. $C_{15}H_{17}NO_7$ requires *M*, 323.1005); δ_H(250 MHz; [$^2H_6$]DMSO + CD_3CO_2D), 1.5–1.7 (6H, m, $CH_2CH_2CH_2$), 3.74 (1H, dd, *J* 2, 5, CHOH), 4.67 (1H, m, $CHOCONH_2$), 4.81 (1H, d, *J* 15, OCH_AH_B), 4.99 (1H, d, *J* 15, OCH_AH_B), 5.33 (1H, d, *J* 5, OH), 6.36 (2H, br s, NH_2), 6.72 (1H, d, *J* 8, Ar), 7.70 (1H, d, *J* 1, Ar), 7.75 (1H, dd, *J* 1, 8, Ar), 12.5 (1H, br s, CO_2H); *m/z* (CI^+) 324 (MH^+, 100%).

(2*R,3a′*R**,7a′*R**)-6-(2-Formylphenyl)-5′,5′-dimethylspiro-[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxol]-2′-one 35**

A mixture of the boronic ester **34b** (917 mg, 4.83 mmol), **22b** (1.19 g, 3.22 mmol) and caesium carbonate (1.63 g, 5. mmol) was stirred in DMF (25 ml) under an atmosphere of argon. Palladium tetrakis(triphenylphosphine) (75 mg, 0.06 mmol) was added and the reaction mixture heated at 80 °C for 4 h. The mixture was cooled to room temperature, diluted with CH_2Cl_2 (30 ml) and water (30 ml) and separated. The organic layer was dried over $MgSO_4$ and then concentrated under vacuum and chromatographed on silica using 20–30% EtOAc–hexane as eluent to give **35** as an off white gum (1.1 g, 87%), mp. 105–112 °C (Found: C, 70.1; H, 5.4. $C_{23}H_{22}O_6$ requires C, 70.0; H, 5.6%); δ_H(200 MHz; [$^2H_6$]DMSO), 1.06 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.5–2.0 [4H, m, $CH_2C(CH_3)_2CH_2$], 5.01 (2H, s, OCH_2), 5.04 (1H, d, *J* 6, $CH_2CHOCHO$), 5.19 (1H, m, $CH_2CHOCHO$), 6.99 [1H, d, *J* 8, ArC(6)H], 7.23 [1H, d, *J* 2, ArC(3)H], 7.29 [1H, dd, *J* 2, 8, ArC(5)H], 7.51 [1H, dt, *J* 1, 7, ArC(4′)H], 7.58 [1H, dd, *J* 1, 7, ArC(6′)H], 7.75 [1H, dt, *J* 2, 7, ArC(5′)H], 7.91 [1H, dd, *J* 2, 7, ArC(3′)H], 9.92 (1H, s, CHO); *m/z* (CI^+) 395 (MH^+, 100%).

(2*R,3a′*R**,7a′*R**)-6-(2-Carboxyphenyl)-5′,5′-dimethylspiro-[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxol]-2′-one 36**

The aldehyde **35** (159 mg, 0.4 mmol) was dissolved in THF (3 ml) and sulfamic acid (52 mg, 0.53 mmol) was added along with water (2 ml). The mixture was stirred for 5 min and then sodium chlorite (47 mg, 0.52 mmol) was added and the reaction mixture was stirred for 30 min at room temperature. EtOAc (5 ml) and water (5 ml) were added and the organic layer separated, dried over $MgSO_4$, filtered and concentrated under vacuum to give the acid **36** as a colourless gum (162 mg, 98%), δ_H(200 MHz; [$^2H_6$]DMSO), 1.08 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.5–2.0 [4H, m, $CH_2C(CH_3)_2CH_2$], 4.99 (2H, s, OCH_2), 4.96 (1H, d, *J* 6, $CH_2CHOCHO$), 5.18 (1H, m, $CH_2CHOCHO$), 6.90 [1H, d, *J* 8, ArC(6)H], 7.12 [1H, d, *J* 2, ArC(3)H], 7.19 [1H, dd, *J* 2, 8, ArC(5)H], 7.37 [1H, dd, *J* 2, 7, ArC(6′)H], 7.43 [1H, dt, *J* 1, 7, ArC(4′)H], 7.55 [1H, dt, *J* 2, 7, ArC(5′)H], 7.73 [1H, dd, *J* 2, 7, ArC(3′)H]; *m/z* (CI^+) 411 (MH^+, 100%).

(2*R,2′*R**,3′*R**)-3′-Carbamoyloxy-6-(2-carboxyphenyl)-2′-hydroxy-5′,5′-dimethylspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 13a and (2*R**,2′*R**,3′*R**)-2′-carbamoyloxy-6-(2-carboxyphenyl)-3′-hydroxy-5′,5′-dimethylspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 37a**

The carbamates **13a** + **37a** were prepared from **36** (158 mg, 0.38 mmol) and ethanolic ammonia in an analogous fashion to that described for **13b** and **37b** except that the 1:1 mixture of isomers that were produced was not separated (120 mg, 73%). Satisfactory analyses were obtained with spectral properties similar to those of **13b** and **37b**.

(2*R,2′*R**,3′*R**)-6-(2-Carboxyphenyl)-2′-hydroxy-5′,5′-dimethyl-3′-methylcarbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 13b and (2*R**,2′*R**,3′*R**)-6-(2-carboxyphenyl)-3′-hydroxy-5′,5′-dimethyl-2′-methylcarbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 37b**

The carbonate **36** (250 mg, 0.61 mmol) was stirred in a saturated solution of methylamine (30% in industrial methylated spirits) for 18 h and then diluted with water (10 ml), adjusted to pH 2 with conc. HCl and extracted with EtOAc (10 ml). The

organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum to give **13b** and **37b** in a 2:1 mixture as an oil. Et_2O was added and **37b** was precipitated as a white solid which was collected by filtration (39 mg, 15%). The filtrate which still contained a mixture of **13b** and **37b** was chromatographed on silica using 0.1% HOAc–70–100% EtOAc–hexane as eluent to give a pure sample of **13b** (144 mg, 53%). **13b** (R_f 0.1% HOAc–EtOAc 0.2) (Found: M^+, 441.1760. $C_{24}H_{27}NO_7$ requires *M*, 441.1787); δ_H(250 MHz; [$^2H_6$]DMSO), 0.93 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.3–1.9 [4H, m, $CH_2C(CH_3)_2CH_2$], 3.88 (1H, m, CHOH), 4.77 (1H, d, *J* 15, OCH_ACH_B), 4.91 (1H, d, *J* 15, OCH_ACH_B), 4.99 (1H, m, CHOCONHMe), 5.27 (1H, d, *J* 6, CHOH), 6.82 [1H, d, *J* 8, ArC(6)H], 6.90 (1H, m, NH), 7.03 [1H, d, *J* 2, ArC(3)H], 7.12 [1H, dd, *J* 2, 8, ArC(5)H], 7.36 [1H, dd, *J* 2, 7, ArC(6′)H], 7.40 [1H, dd, *J* 2, 7, ArC(4′)H], 7.53 [1H, dt, *J* 1, 7, ArC(5′)H], 7.68 [1H, dt, *J* 2, 7, ArC(5′)H]; *m/z* (ESP^+) 442 (MH^+, 100%). **37b** (R_f 0.1% HOAc–EtOAc 0.15) (Found: M^+, 441.1745. $C_{24}H_{27}NO_7$ requires *M*, 441.1787); δ_H(250 MHz; [$^2H_6$]DMSO), 0.95 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.3–1.7 [4H, m, $CH_2C(CH_3)_2CH_2$], 2.58 (3H, d, *J* 4, $NHCH_3$), 4.00 (1H, m, CHOH), 4.70 (1H, d, *J* 6, CHOCONH-CH_3), 4.73 (1H, d, *J* 15, OCH_ACH_B), 4.94 (1H, d, *J* 15, OCH_A-CH_B), 5.06 (1H, m, CHOH), 6.84 [1H, d, *J* 8, ArC(6)H], 6.97 (1H, m, NH), 7.03 [1H, d, *J* 2, ArC(3)H], 7.13 [1H, dd, *J* 2, 8, ArC(5)H], 7.33 [1H, dd, *J* 2, 7, ArC(6′)H], 7.41 [1H, dd, *J* 2, 7, ArC(4′)H], 7.53 [1H, dt, *J* 1, 7, ArC(5′)H], 7.67 [1H, dt, *J* 2, 7, ArC(5′)H]; *m/z* (ESP^+) 442 (MH^+, 70%), 216 ($MH^+ - C_{10}H_{17}NO_4$, 100).

(2*R,2′*R**,3′*R**)-6-(2-Carboxyphenyl)-2′-hydroxy-5′,5′-dimethyl-3′-ethylcarbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 13c and (2*R**,2′*R**,3′*R**)-6-(2-carboxyphenyl)-3′-hydroxy-5′,5′-dimethyl-2′-ethylcarbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 37c**

The carbamates **13c** + **37c** were prepared from **36** (250 mg, 0.61 mmol) and ethylamine in ethanol in an analogous fashion to that described for **13b** and **37b** to give **13c** (158 mg, 57%) as a gum and **37c** as a white solid (68 mg, 25%). Satisfactory analyses were obtained with spectral properties similar to those of **13b** and **37b**.

(2*R,2′*R**,3′*R**)-6-(2-Carboxyphenyl)-2′-hydroxy-5′,5′-dimethyl-3′-isopropylcarbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 13d and (2*R**,2′*R**,3′*R**)-6-(2-carboxyphenyl)-3′-hydroxy-5′,5′-dimethyl-2′-isopropylcarbamoyloxyspiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 37d**

The carbamates **13d** + **37d** were prepared from **36** (250 mg, 0.61 mmol) and ethylamine in ethanol in an analogous fashion to that described for **13b** and **37b** to give **13d** (195 mg, 68%) as a gum and **37d** as a white solid (55 mg, 19%). Satisfactory analyses were obtained with spectral properties similar to those of **13b** and **37b**.

(3*R,3a′*R**,7a′*R**)- and (2*S**,3a′*R**,7a′*R**)-6-Bromo-2′,2′,6′,6′-tetramethylspiro[4*H*-1,3-benzodioxine-2,4′-perhydro-[1,3]benzodioxole] 38 and 39**

A mixture of the diols **22b** and **23b** (6.6 g, 19.2 mmol) was dissolved in DMF (50 ml) and 2,2-dimethoxypropane (10 ml) was added along with conc. HCl (0.2 ml). The solution was stirred for 18 h at room temperature and then partitioned between EtOAc (150 ml) and water (150 ml). The organic layer was washed with water (2 × 100 ml) and brine (100 ml), dried over $NaSO_4$, filtered and concentrated. The residual oil was chromatographed on silica using 3–6% EtOAc–hexane as eluent to give **38** (3.2 g, 43%) as a colourless oil and **39** (3.1 g, 42%) as a white solid. **38** (Found: C, 56.9; H, 5.9. $C_{18}H_{23}BrO_4$ requires C, 56.4; H, 6.05%); δ_H(400 MHz; $CDCl_3$) 1.015 (3H, s, CH_2-$CCH_{3ax}CH_{3eq}$), 1.08 (3H, s, $CH_2CCH_{3ax}CH_{3eq}$), 1.34 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.635 (1H, dd, *J* 7, 14, $CH_{eq}CH_{ax}$-CHO), 1.75 (1H, dd, *J* 5, 14, $CH_{eq}CH_{ax}CHO$), 1.78 [2H, s, $CH_2C(OR)_2$], 4.185 (1H, d, *J* 5.5, $CH_2CHOCHO$), 4.50 (1H, dd, *J* 2, 6, $CH_2CHOCHO$), 4.82 (1H, d, *J* 15, OCH_AH_B), 4.98 (1H, d, *J* 15, OCH_AH_B), 6.70 (1H, d, *J* 9, ArH), 7.125 (1H, d, *J* 2, ArH), 7.265 (1H, dd, *J* 2, 9, ArH); irradiation of CH_{3ax} (1.015) gives an NOE enhancement of ArH (6.70); *m/z* (CI^+) 383 (MH^+, 70%), 202 ($C_7H_5OBr + NH_4^+$, 100). **39**, Mp 66–75 °C (Found: C, 56.7; H, 6.2. $C_{18}H_{23}BrO_4$ requires C, 56.4; H, 6.05%); δ_H(400 MHz; $CDCl_3$) 1.04 (3H, s, $CH_2CCH_{3ax}CH_{3eq}$), 1.07 (3H, s, $CH_2CCH_{3ax}CH_{3eq}$), 1.35 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.67–1.85 [4H, m, $CH_2C(CH_3)_2CH_2$], 4.155 (1H, dd, *J* 1, 6, $CH_2CHOCHO$), 4.485 (1H, dd, *J* 7, 13, $CH_2CHOCHO$), 4.83 (2H, s, OCH_2), 6.815 (1H, d, *J* 9, ArH), 7.075 (1H, d, *J* 2, ArH), 7.25 (1H, dd, *J* 2, 9, ArH); irradiation of CH_{3ax} (1.04) gives an NOE enhancement of OCH_2 (4.83); *m/z* (CI^+) 383 (MH^+, 20%), 216 ($C_{11}H_{18}O_3 + NH_4^+$, 100).

(2*R,3a′*R**,7a′*R**)-6-(1,3,2-Dioxaborinan-1-yl)-2′,2′,6′,6′-tetramethylspiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]-benzodioxole] 40**

38 (3.1 g, 8.1 mmol) was dissolved in THF (80 ml) and cooled under argon in a dry ice–acetone bath. Bu^{n}Li (1.6 M in hexane; 5.6 ml, 8.9 mmol) was added over 10 min and the reaction mixture stirred for a further 5 min before adding triisopropyl borate (1.82 g, 9.7 mmol) rapidly. The mixture was allowed to warm to room temperature, diluted with saturated aqueous ammonium chloride (100 ml) and extracted with EtOAc (100 ml). The organic layer was washed with water (2 × 50 ml) and concentrated under vacuum. Toluene (100 ml) was added followed by propane-1,3-diol (5 ml) and the mixture stirred for 1 h at room temperature, diluted with water and the organic layer concentrated under vacuum to give **40** (2.6 g, 80%) as a pale yellow oil which crystallised on standing, mp 125–127 °C (Found: C, 65.2; H, 7.5. $C_{21}H_{29}BO_6$ requires C, 65.0; H, 7.5%); δ_H(200 MHz; $CDCl_3$) 1.03 (3H, s, CH_{3ax}), 1.07 (3H, s, CH_{3eq}), 1.33 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.6–1.8 (2H, m, CH_2CHO), 1.81 [2H, s, $CH_2C(OR)_2$], 2.03 [2H, quintet, *J* 5, $CH_2(CH_2O)_2B$], 4.14 [4H, t, *J* 5, $CH_2(CH_2O)_2B$], 4.20 (1H, d, *J* 6.5, $CH_2CHOCHO$), 4.49 (1H, m, $CH_2CHOCHO$), 4.85 (1H, d, *J* 15, OCH_AH_B), 5.02 (1H, d, *J* 15, OCH_AH_B), 6.78 (1H, d, *J* 8, ArH), 7.41 (1H, d, *J* 2, ArH), 7.56 (1H, dd, *J* 2, 8, ArH); *m/z* (EI^+) 388 (M^+, 20%), 330 ($M^+ - C_3H_6O$, 20), 190 ($C_{11}H_{18}O_3^+$, 100).

(2*R,2′*R**,3′*R**)-2′,3′-Dihydroxy-5′,5′-dimethyl-6-(2-pyridyl)-spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 42**

Boronic ester **40** (339 mg, 0.87 mmol) and 2-bromopyridine (138 mg, 0.87 mmol) were dissolved in DMF (10 ml). Cs_2CO_3 (425 mg, 1.3 mmol) was added and the mixture placed under an atmosphere of argon. $Pd(PPh_3)_4$ (20 mg, 0.017 mmol) was added and the mixture stirred at 100 °C for 2 h and then cooled to room temperature. The reaction mixture was partitioned between EtOAc (40 ml) and water (40 ml) and the organic layer was washed with water (2 × 20 ml) and brine (20 ml), dried over Na_2SO_4, filtered and concentrated to give crude **41** (385 mg) as an oil which was dissolved in MeOH (15 ml) and 1 M HCl (3 ml) was added. The solution was stirred for 3 h at room temperature, left standing at 0 °C for 15 h and then diluted with water 20 ml) and adjusted to pH 11 with 1 M NaOH. The aqueous solution was extracted with EtOAc (2 × 30 ml) and the combined organics were washed with brine (20 ml), dried over Na_2SO_4, filtered and concentrated to an oil. Trituration with Et_2O gave a small quantity of **42** as a white crystalline solid (55 mg, 18%) along with the filtrate which was concentrated to an oil (250 mg) containing mostly **42** which was progressed without further purification. For the solid, mp 123–129 °C (Found: C, 69.7; H, 6.8; N, 3.7; H_2O, 0.6. $C_{20}H_{23}NO_4 \cdot 0.1H_2O$ requires C, 70.0; H, 6.8; N, 4.1; H_2O, 0.5%); δ_H(250 MHz; $CDCl_3$) 0.98 (3H, s, CH_{3ax}), 1.02 (3H, s, CH_{3eq}), 1.50–1.72 [4H, m, $CH_2C(CH_3)_2CH_2$], 2.08 (1H, d, *J* 9, CH_2CHOH), 2.45 [1H, s, CHOHC-$(OR)_2$], 3.97 [1H, s, $CHOH(OR)_2$], 4.18 (1H, m, CH_2CHOH),

4.92 (1H, d, *J* 15, OCH_AH$_B$), 5.02 (1H, d, *J* 15, OCH$_A$$H_B$), 6.90 [1H, d, *J* 8, Ar(6)H], 7.19 [1H, m, pyr(5)H], 7.6–7.8 [4H, m, Ar(3 + 5)H + pyr(3 + 4)H], 8.65 [1H, m, pyr(6)H]; *m/z* (ESP$^+$) 342 (MH$^+$, 90%), 184 (100, MH$^+$ − $C_8H_{14}O_3$).

(2*R,3a′*R**,7a′*R**)-6′,6′-Dimethyl-6-(2-pyridyl)spiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxol]-2′-one 43**

The compound was prepared from crude **42** in a similar manner as described for **22a** (*ca.* 44% yield from **40**) which was used directly, δ_H(250 MHz; $CDCl_3$) 1.11 (3H, s, CH_{3ax}), 1.13 (3H, s, CH_{3eq}), 1.8–2.05 [4H, m, CH_2C(CH_3)$_2$CH_2], 4.75 (1H, dd, *J* 1, 8, C*H*O), 4.95 (1H, d, *J* 15, OCH_AH$_B$), 5.0 (1H, m, CHO), 5.08 (1H, d, *J* 15, OCH$_A$$H_B$), 6.91 [1H, d, *J* 8, Ar(6)H], 7.20 [1H, m, pyr(5)H], 7.6–7.8 [4H, m, Ar(3 + 5)H + pyr(3 + 4)H], 8.65 [1H, m, pyr(6)H].

2*R,3a′*R**,7a′*R**)-6′,6′-Dimethyl-6-(1-oxido-2-pyridinio)-spiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxol]-2′-one 44**

50% *m*-Chloroperbenzoic acid (125 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (2 ml), and the organic layer added to a solution of **43** (110 mg, 0.3 mmol) in CH_2Cl_2 (10 ml). The solution was stirred at room temperature for 15 h and then concentrated and chromatographed on silica using 5% MeOH–CH_2Cl_2 as eluent to give **44** as a colourless oil (105 mg, 91%) which was used directly, δ_H(250 MHz; $CDCl_3$) 1.12 (3H, s, CH_{3ax}), 1.13 (3H, s, CH_{3eq}), 1.75–2.0 [4H, m, CH_2C(CH_3)$_2$CH_2], 4.75 (1H, dd, *J* 1, 8, C*H*O), 4.91 (1H, d, *J* 15, OCH_AH$_B$), 5.0 (1H, m, CHO), 5.08 (1H, d, *J* 15, OCH$_A$$H_B$), 6.92 [1H, d, *J* 8, Ar(6)H], 7.21 [1H, dt, *J* 1, 6, pyr(4)H], 7.28 [1H, t, *J* 6, pyr(5)H], 7.60 [1H, dd, *J* 1, 8, Ar(5)H], 7.65 [1H, d, *J* 1, Ar(3)H], 8.30 [1H, d, *J* 6, pyr(6)H]; *m/z* (ESP$^+$) 384 (MH$^+$, 100%).

(2*R,2′*R**,3′*R**)3′-Carbamoyloxy-2′-hydroxy-5′,5′-dimethyl-6-(1-oxido-2-pyridinio)spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 45 and (2*R**,2′*R**,3′*R**)-2′-carbamoyloxy-3′-hydroxy-5′,5′-dimethyl-6-(1-oxido-2-pyridinio)spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 46**

The compounds were prepared as a 1:1 mixture from **44** in a similar manner as described for **24**/**25** (26 mg, 50%) (Found: M$^+$, 400.1625. $C_{21}H_{24}N_2O_6$ requires *M*, 400.1634); δ_H(200 MHz; $CDCl_3$) (**45**, major isomer) 1.04 (3H, s, CH_{3ax}), 1.14 (3H, s, CH_{3eq}), 1.55–1.9 [4H, m, CH_2C(CH_3)$_2$CH_2], 4.1 (1H, br s, C*H*OH), 4.73 (1H, br s, OH), 4.9 (2H, m, OC$H_A$$H_B$), 5.25 (1H, m, C*H*OCONH$_2$), 6.90 [1H, d, *J* 8, Ar(6)H], 7.3–7.75 (7H, m, 5 × ArH + NH$_2$), 8.55 [1H, dd, *J* 0.5, 8, pyr(6)H]; (**46**, minor isomer) 1.01 (3H, s, CH_{3ax}), 1.11 (3H, s, CH_{3eq}), 1.5–1.9 [4H, m, CH_2C(CH_3)$_2$CH_2], 4.55 (1H, m, C*H*OH), 4.74 (1H, br s, OH), 4.9 (2H, m, OC$H_A$$H_B$), 5.24 (1H, br s, C*H*OCONH$_2$), 6.95 [1H, d, *J* 8, Ar(6)H], 7.3–7.75 (7H, m, 5 × ArH + NH$_2$), 8.55 [1H, dd, *J* 0.5, 8, pyr(6)H]; *m/z* (ESP$^+$) 401 (MH$^+$, 95%), 200 (100, M$^+$ − $C_9H_{15}NO_4$).

(2*R,2′*R**,3′*R**)-2-Hydroxy-5′,5′-dimethyl-3′-methylcarbamoyloxy-6-(1-oxido-2-pyridinio)spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 47 and (2*R**,2′*R**,3′*R**)-3-hydroxy-5′,5′-dimethyl-2′-methylcarbamoyloxy-6-(1-oxido-2-pyridinio)-spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 48**

The compounds were prepared as a 2:1 mixture from **44** in a similar manner as described for **24**/**25** except using methylamine (44 mg, 82%) (Found: M$^+$, 414.1794. $C_{22}H_{26}N_2O_6$ requires *M*, 414.1791); δ_H(400 MHz; $CDCl_3$) (**47**, major isomer) 1.01 (3H, s, CH_{3ax}), 1.12 (3H, s, CH_{3eq}), 1.5–1.9 [4H, m, CH_2C(CH_3)$_2$CH_2], 2.81 (3H, d, *J* 5, NHCH_3), 4.1 (1H, br s, C*H*OH), 4.79 (1H, br s, OH), 4.92 (1H, d, *J* 15, OCH_AH$_B$), 4.99 (1H, d, *J* 15, OCH$_A$$H_B$), 5.29 (1H, m, C*H*OCONHMe), 6.98 [1H, d, *J* 8, Ar(6)H], 7.23 + 7.31 + 7.44 [3 × 1H, m, pyr(3,4,5)H], 7.55 [1H, dd, *J* 2, 7, Ar(5)H], 7.77 [1H, d, *J* 2, Ar(3)H], 8.33 [1H, d, *J* 8, pyr(6)H]; (**48**, minor isomer) 1.04 (3H, s, CH_{3ax}), 1.15 (3H, s, CH_{3eq}), 1.5–1.9 [4H, m, CH_2C(CH_3)$_2$CH_2], 2.87 (3H, d, *J* 5, NHCH_3), 4.3 (1H, m, C*H*OH), 4.79 (1H, br s, OH), 4.85 (1H, d, *J* 15, OCH_AH$_B$), 4.95 (1H, d, *J* 15, OCH$_A$$H_B$), 5.24 (1H, br s, C*H*OCONHMe), 6.94 [1H, d, *J* 8, Ar(6)H], 7.23 + 7.31 + 7.44 [3 × 1H, m, pyr(3,4,5)H], 7.63 [1H, dd, *J* 2, 7, Ar(5)H], 7.77 [1H, m, Ar(3)H], 8.33 [1H, d, *J* 8, pyr(6)H]; *m/z* (ESP$^+$) 415 (MH$^+$, 85%), 200 (100, M$^+$ − $C_{10}H_{17}NO_4$).

(2*R,3a′*R**,7a*R**)-2′,2′,6′,6′-Tetramethyl-6-[2-(1,1,1-trichloroethoxycarbonyl)phenyl]spiro[4*H*-1,3-benzodioxine-2,4′-perhydro[1,3]benzodioxole] 49**

This compound was prepared from **40** and 1,1,1-trichloroethyl 2-iodobenzoate in a similar manner as described for **41** (420 mg, 53%), mp 57–58 °C (Found: C, 58.6; H, 5.5. $C_{27}H_{29}C_{13}O_6$ requires C, 58.3; H, 5.3%); δ_H(200 MHz; $CDCl_3$) 1.05 (6H, s, CH_{3ax} + CH_{3eq}), 1.26 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.55–-1.8 [4H, m, CH_2C(CH_3)$_2$CH_2], 4.15 (1H, dd, *J* 1, 6.5, CH_2CHOC*H*O), 4.46 (1H, m, CH_2C*H*OCHO), 4.76 (1H, d, *J* 15, OCH_AH$_B$), 4.90 (2H, s, CO_2CH_2) 4.94 (1H, d, *J* 15, OCH$_A$$H_B$), 6.82 (1H, d, *J* 8, ArH), 7.05 (1H, d, *J* 2, ArH), 7.13 (1H, dd, *J* 2, 8, Ar′H), 7.43 (1H, dd, *J* 2, 8, ArH), 7.51 (1H, dt, *J* 2, 8, Ar′H), 7.65 (1H, dt, *J* 2, 8, Ar′H), 7.83 (1H, dd, *J* 2, 8, Ar′H); *m/z* (CI$^+$) 555 (M$^+$, 100%).

(2*R,2′*R**,3′*R**)-(2′,3′-Dihydroxy)-5′,5′-dimethyl-6-[2-(1,1,1-trichloroethoxycarbonyl)phenyl]spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 50**

The acetonide **49** (360 mg, 0.65 mmol) was dissolved in acetonitrile (10 ml) and HCl (1 M; 3 ml) was added. The solution was stirred for 2 h at room temperature and then quenched by the addition of solid $NaHCO_3$. Water (10 ml) was added and the aqueous solution was extracted with EtOAc (30 ml) and the organic layer washed with brine (20 ml), dried over Na_2SO_4, filtered and concentrated under vacuum to give **50** (350 mg) as a slightly impure colourless oil which was used directly, δ_H(200 MHz; $CDCl_3$) 0.92 (3H, s, CH_{3ax}), 1.05 (3H, s, CH_{3eq}), 1.25 (1H, m, CH_{eq}H$_{ax}$CHOH), 1.46–1.74 [3H, m, CH_2C(CH_3)$_2$C$H_{eq}$$H_{ax}$-CHOH], 3.70 (1H, m, CH_2CHOHC*H*OH), 3.85 (1H, m, CH_2C*H*OHCHOH), 4.38 (1H, d, *J* 7, OH), 4.49 (1H, d, *J* 6, OH), 4.75 (1H, d, *J* 15, OCH_AH$_B$), 4.89 (1 H, d, *J* 15, OCH$_A$$H_B$), 4.90 (2H, s, CO_2CH_2) 6.80 (1H, d, *J* 8, ArH), 7.02 (1H, d, *J* 2, ArH), 7.12 (1H, dd, *J* 2, 8, Ar′H), 7.43 (1H, dd, *J* 2, 8, ArH), 7.51 (1H, dt, *J* 2, 8, Ar′H), 7.65 (1H, dt, *J* 2, 8, Ar′H), 7.84 (1 H, dd, *J* 2, 8, Ar′H); *m/z* (ESP$^+$) 532 (M + NH_4^+, 100%).

(2*R,2′*R**,3′*R**)-2′-Hydroxy-5′,5′-dimethyl-3′-(5-methylpyrrol-2-ylcarbonyloxy)-6-[2-(1,1,1-trichloroethoxycarbonyl)-phenyl]spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 51**

The diol (**50**, 284 mg, 0.55 mmol) and 5-methyl-2-trichloroacetylpyrrole (375 mg, 1.65 mmol) were dissolved in THF (30 ml) and potassium *tert*-butoxide (74 mg, 0.66 mmol) was added. The mixture was stirred at reflux for 2 h and further portions of the pyrrole (200 mg, 0.88 mmol) and potassium *tert*-butoxide (80 mg, 0.71 mmol) were added. After stirring at reflux for a further 2 h more potassium *tert*-butoxide (100 mg, 0.89 mmol) was added and the mixture stirred at reflux for 15 h, cooled and diluted with NH_4Cl (sat. aq., 30 ml) and extracted with EtOAc (30 ml). The organic layer was washed with brine (20 ml), dried over Na_2SO_4, filtered and concentrated under vacuum to a yellow oil which was chromatographed on silica using 1:2 EtOAc–hexane as eluent to give **51** as a colourless gum (73 mg, 21%) along with recovered **50** (57 mg, 27% yield of **51** based on recovered starting material). **51** (Found: M$^+$, 621.1034. $C_{30}H_{30}NO_7Cl_3$ requires *M*, 621.1088); δ_H(250 MHz, $CDCl_3$) 1.02 (3H, s, CH_{3ax}), 1.15 (3H, s, CH_{3eq}), 1.2–1.75 [4H, m, CH_2C(CH_3)$_2$CH_2], 2.30 (3H, s, pyrrCH_3), 4.14 (1H, m, C*H*OH), 4.75 (2H, s, OCH_2), 4.90 (2H, s, CO_2CH_2), 5.52 (1H, m, C*H*OCO), 5.95 (1H, m, pyrrC3-H), 6.81 (1H, m, pyrrC4-H), 6.90 (1H, d, *J* 8, ArH), 6.95 (1H, d, *J* 2, ArH), 7.14 (1H, dd, *J* 2, 8, Ar′H), 7.35 (1H, dd, *J* 2, 8, ArH), 7.42

(1H, dt, *J* 2, 8, Ar′H), 7.60 (1H, dt, *J* 2, 8, Ar′H), 7.93 (1H, dd, *J* 2, 8, Ar′H), 9.0 (1H, br s, NH); *m/z* (CI^+) 622 ($M + H^+$, 100%).

(2*R,2′*R**,3′*R**)-6-(2-Carboxyphenyl)-2′-hydroxy-5′,5′-dimethyl-3′-(5-methylpyrrol-2-carbonyloxy)spiro[4*H*-1,3-benzodioxine-2,1′-cyclohexane] 52**

The trichloroethyl ester **51** (73 mg, 0.117 mmol) was dissolved in THF (5 ml). Zinc dust (250 mg) was added along with NH_4OAc (1M; 6 ml) and acetic acid (5 ml). The mixture was stirred at room temperature for 2 h and then filtered through Celite, washing the residue with water (5 ml) and EtOAc (10 ml). A further portion of EtOAc (20 ml) was added and the organic layer separated and washed with water (10 ml) and brine (10 ml), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was chromatographed on silica using 7–12% MeOH–CH_2Cl_2 as eluent, to afford **52** (25 mg, 44%) as a colourless glassy solid (Found: M^+, 491.1906. $C_{28}H_{29}NO_7$ requires *M*, 491.1944); δ_H(250 MHz; $CDCl_3$) 0.98 (3H, s, CH_{3ax}), 1.11 (3H, s, CH_{3eq}), 1.2–1.95 [4H, m, $CH_2C(CH_3)_2CH_2$], 2.20 (3H, s, pyrrCH_3), 4.15 (1H, m, C*H*OH), 4.85 (2H, s, OCH_2), 5.46 (1H, m, C*H*OCO), 5.93 (1H, s, pyrrC3-H), 6.75–6.85 (3H, m, pyrrC4-H + 2 × ArH), 7.13 (1H, d, *J* 8, Ar′H), 7.30–7.40 (2H, m, ArH + Ar′H), 7.50 (1H, t, *J* 8, Ar′H), 7.86 (1H, d, *J* 8, Ar′H), 9.4 (1H, br s, NH); *m/z* (ESP^+) 492 ($M + H^+$, 100%).

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