Cyclisation of Novel Amino Oxo Esters to Tetramic Acids − Density Functional Theory Study of the Reaction Mechanism

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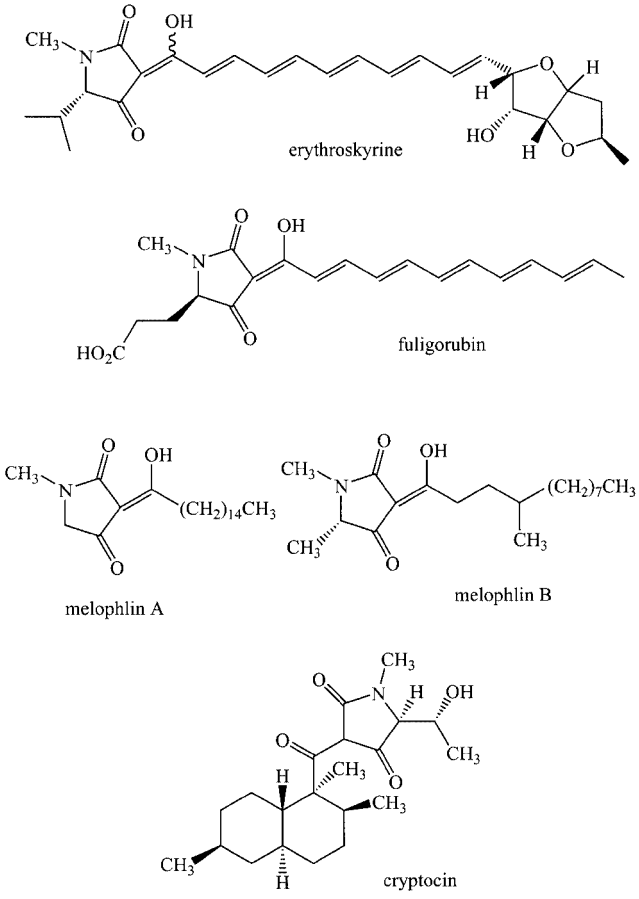
The synthesis of novel *N*-urethane-protected γ-methylamino-β-oxo esters and their use as precursors for the preparation of *N*-methyltetramic acids is described. The presence of the bulky urethane protecting group on the nitrogen atom gives rise to rotational isomers detectable in the NMR spectra of the compounds, along with the keto/enol tautomerism. The mechanism of the cyclisation reaction of γ-amino-β-oxo esters to tetramic acids was studied theoretically by the B3LYP hybrid density functional method.

Introduction

The pyrrolidine-2,4-dione ring system (tetramic acid) is present in various naturally occurring compounds exhibiting a wide range of biological and pharmaceutical activities.[1] Representative examples are erythroskyrine[2] [a mycotoxin containing an *N*-methyl-(*S*)-valine-derived acyltetramic acid terminus] and fuligorubin A[3] [a pigment from the slime mould *Fuligo septica (L.)* bearing a polyene chromophore]. Recently, several natural products incorporating the *N*-methyltetramic acid nucleus have been isolated. These include aflastatin A,[4] a specific inhibitor of aflatoxin production by *Asp. parasiticus*, vancoresmycin,[5] a new antibiotic active against Gram-positive bacteria, melophlins A and B,[6] which exhibit anticancer activity, and melophlins C−O with antibacterial and antifungal activity,[7] ancorinoside A and its Mg salt,[8] and the potent antimycotic agent cryptocin[9] (Scheme 1).

Since we have long been involved in the development of a new methodology for the synthesis of *N*-acyl- and *N*-urethane-protected tetramic acids through *C*-acylation/cyclisation reactions of active methylene compounds with α-amino acid activated derivatives as acylating agents,[10] we focused on the extension of this synthetic route to the preparation of *N*-methyltetramic acids, using the *N*-hydroxysuccinimidyl ester of *tert*-butoxycarbonyl-*N*-methylglycine (Boc-sarcosine) as acylating agent. Removal of the Boc-protecting group from the *C*-acylation compounds and subsequent cyclisation of the corresponding hydrochloride



Scheme 1. Examples of natural products incorporating the *N*-methyltetramic acid nucleus

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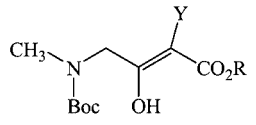
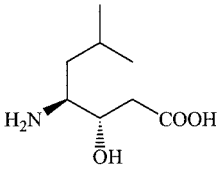
salts under basic conditions provided the desired *N*-methyltetramic acids.

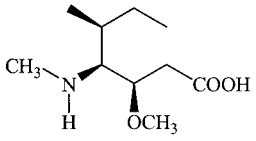
One interesting feature of this synthetic route is the isolation of the *C*-acylation compounds, 4-[Boc(methyl)amino]-3-hydroxybutenoates (Scheme 2), which belong to the structural class of γ-amino-β-hydroxy acids and are novel statine precursors. Statine (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, and its analogues (Scheme 2) are widely used in the design and synthesis of pseudopeptides that inhibit aspartic acid proteases, such as renin, pepsin and cathepsin D.[11] The *N*-methyl group as present in 4-[Boc(methyl)amino]-3-hydroxybutenoates can also be found in the statine analogue dolaisoleuine [(3*R*,4*S*,5*S*)-*N*,*O*-dimethylisostatine] (Scheme 2), a component of dolastatin 10, a marine natural product with cytotoxic and antineoplastic activity.[12] The synthesis of *N*-methylstatine analogues is therefore of particular interest, since they can be useful for structure-activity relationship studies. On the other hand, these compounds can also be used as precursors for the synthesis of 3-substituted *N*-methyltetramic acids.



4-[Boc(methyl)amino]-3-hydroxy-2-butenoates



Statine



Dolaisoleuine

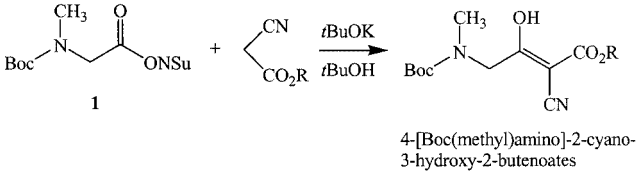
Scheme 2

Furthermore, to investigate possible mechanisms for the cyclisation reactions that produce tetramic acids, we have examined the structures and energetics of critical points on the potential energy surfaces of 2-(alkoxycarbonyl)-4-(alkylamino)-3-hydroxy-2-butenoates by theoretical calculations by the B3LYP Hybrid Density Functional Theory (DFT) method in vacuo and in solvent environments.

Results and Discussion

Experimental Results

The *N*-hydroxysuccinimidyl ester of Boc-sarcosine (**1**) has proved to be a powerful acylating agent when used to acylate alkyl cyanoacetates under basic conditions, providing the corresponding 4-[Boc(methyl)amino]-2-cyano-3-hydroxy-2-butenoates (Scheme 3) in good yields.[13]



4-[Boc(methyl)amino]-2-cyano-3-hydroxy-2-butenoates

Scheme 3

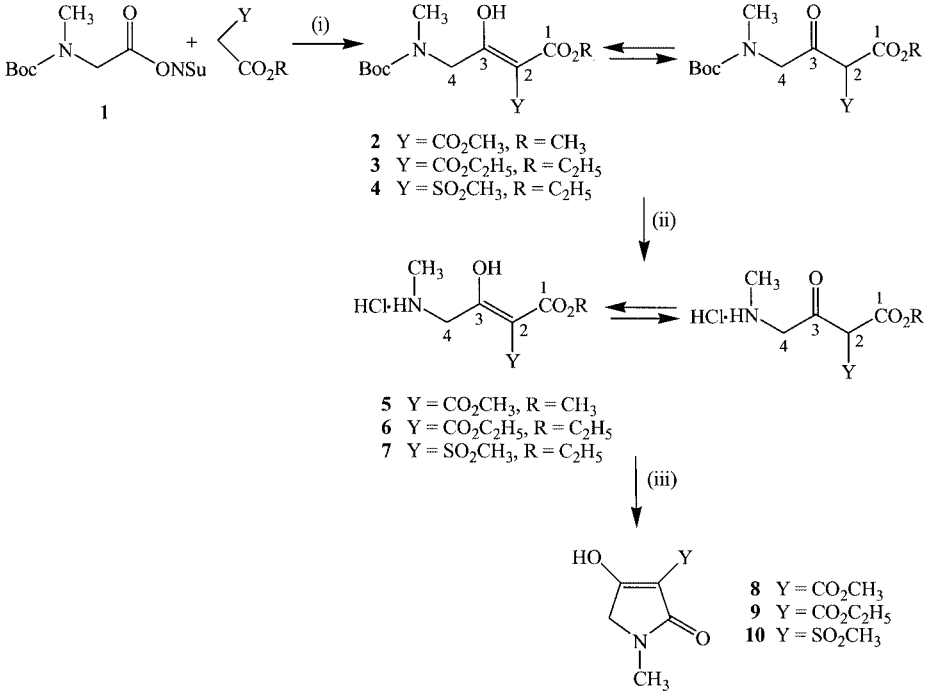
With this in mind, we performed the base-promoted *C*-acylation reactions of dimethyl and diethyl malonate and ethyl methylsulfonyl acetate with the *N*-hydroxysuccinimidyl ester of Boc-sarcosine (**1**) in order to investigate the possibility of isolating the corresponding 2-substituted 4-[Boc(methyl)amino]-3-hydroxy-2-butenoates, which, apart from being novel statine analogues, could be used as precursors for the synthesis of the requisite 3-substituted *N*-methyltetramic acids.

The *N*-hydroxysuccinimidyl ester of Boc-sarcosine (**1**) was prepared by standard experimental procedures.[13] The *C*-acylation reactions were performed under basic conditions with NaH in THF (Scheme 4) and excess of the active methylene compound. The corresponding 2-substituted 4-[Boc(methyl)amino]-3-hydroxy-2-butenoates **2**−**4** were isolated as viscous, colourless oils after flash column chromatography (see Exp. Sect.).

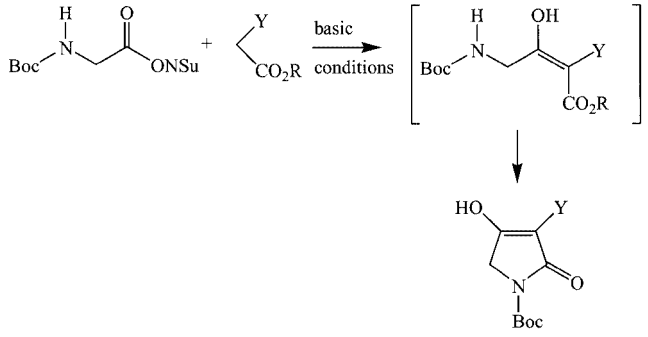
Removal of the Boc-protecting group proved to be essential for the cyclisation of the 2-substituted 4-[Boc(methyl)-amino]-3-hydroxy-2-butenoates to the requisite 3-substituted *N*-methyltetramic acids. This deprotection was effected by treatment with saturated HCl in MeOH or EtOH at room temperature, and the corresponding hydrochloride salts **5**−**7** were isolated as hygroscopic, low-melting, white solids after 0.5 h. Treatment of these salts with RONa/ROH at room temperature for 24 h (monitored by TLC) resulted in the isolation of 3-substituted *N*-methyltetramic acids **8**−**10** in good yields (70−90%).

It is worth noting that, when analogous reactions were performed with the *N*-hydroxysuccinimidyl esters of Boc-, *Z*- and *N*-acetylglycine, the corresponding *C*-acylation compounds could not be isolated and the *N*-substituted tetramic acids were obtained as the only products directly after workup (Scheme 5).[9] This is due to the presence of a proton directly attached to the nitrogen atom. This proton is readily lost under basic conditions to initiate intramolecular cyclisation, as we propose below in our DFT study of the reaction mechanism. The lack of a proton on the nitrogen atom blocks the cyclisation path, so the *C*-acylation products **2**, **3** and **4** can be isolated.

The ^{1}H and ^{13}C NMR spectra of 2-substituted 4-[Boc(methyl)amino]-3-hydroxy-2-butenoates **2**−**4** are of special interest. These molecules are representative examples of β-dicarbonyl compounds capable of undergoing keto/enol tautomerism. Moreover, the presence of the bulky *tert*-butoxycarbonyl protecting group gives rise to rotational isomers for both the keto and the enol tautomers (Scheme 6) as a result of hindered rotation around the C−N bond.



Scheme 4. (i) NaH/THF, 1 h, room temp.; (ii) HCl/ROH, 0.5 h, room temp.; (iii) RONa/ROH, 24 h, room temp.

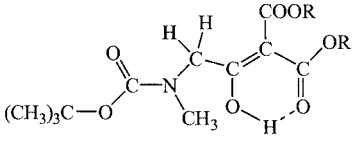


Scheme 5

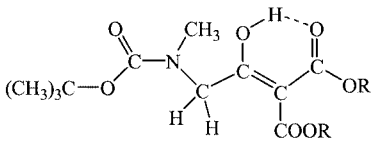
These rotamers are detectable at room temperature, as has been previously reported for *N*,*N*-disubstituted amides[14] and *N*-substituted *N*-methylamino acid derivatives.[12,15]

In $CDCl_3$ solution, compounds **2**−**4** exist as mixtures of keto and enol tautomers. The keto tautomer is detected by the presence of a sharp singlet at δ = 4.5−5.0 ppm, attributable to the methine proton, whereas the two broad singlets at low field (δ = 13−14 ppm) are indicative of the presence of the strongly deshielded proton of the enol form participating in a hydrogen bond.

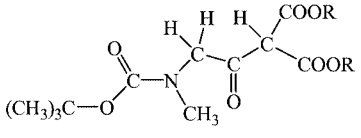
The presence of enol/keto tautomerism along with rotational isomerism gives rise to relatively complex spectra for these compounds. In the ^1H NMR spectrum ($CDCl_3$)



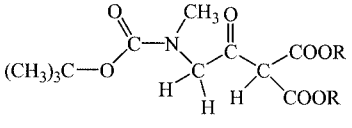
enol *transoid* form



enol *cisoid* form



keto *transoid* form



keto *cisoid* form

Scheme 6. Rotational isomers of 2-substituted 4-[Boc(methyl)amino]-3-hydroxy-2-butenoates

of methyl 4-[(*tert*-butoxycarbonyl)(methyl)amino]-3-hydroxy-2-(methoxycarbonyl)-2-butenoate (**2**), the simplest of the *C*-acylation compounds synthesized, we can clearly observe two sets of signals, one for each tautomer, which are split further into two sets of signals, one for each rotamer.

In this case the enol/keto ratio is 1.2 (based on integration of the hydroxy and methine protons), so the enol tautomer slightly predominates. According to the literature, the protons of the *N*-methyl group *cis* to the oxygen atom (*cisoid* form) should resonate at higher field than those *trans* to the oxygen atom (*transoid* form) because the *cis*-methyl groups experience greater shielding.[14] These facts facilitated the assignment of the signals of the spectrum and allowed the estimation of the *transoid*/*cisoid* ratio in the case of each tautomer. The results are summarized in Table 1.

Table 1. Selected ^{1}H NMR chemical shifts for methyl 4-[(*tert*-butoxycarbonyl)(methyl)amino]-3-hydroxy-2-(methoxycarbonyl)-2-butenoate (**2**) (300 MHz, $CDCl_3$)

	Enol tautomer		Keto tautomer	
	transoid form	*cisoid* form	*transoid* form	*cisoid* form
$N-CH_3$	2.87	2.85	2.91	2.89
transoid/*cisoid*	1.1		1.2	
$-CH_2-$	4.18	4.12	4.34	4.26
transoid/*cisoid*	1.1		1.2	

It can clearly be observed that in both cases (enol and keto tautomers) the *transoid* rotamer is slightly more abundant than the *cisoid* form. This observation is consistent with the fact that, since the equilibrium distributions of *cis* and *trans* isomers of unsymmetrical *N*,*N*′-disubstituted amides depend on both steric and electronic factors, the more stable rotamers should be those in which the bulkier *N*-substituents are *cis* to the carbonyl oxygen atoms (the *transoid* forms in this case).

Doubling of the corresponding signals of most of the carbon atoms in the ^{13}C NMR spectra of compounds **2**−**4** was also observed.

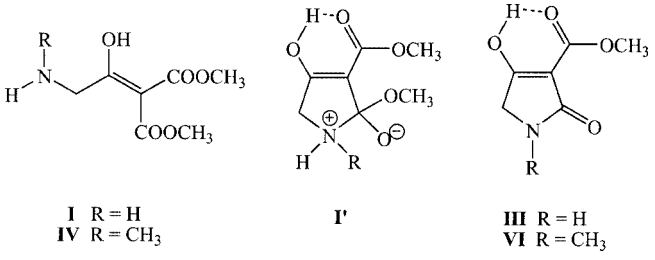
The hydrochloride salts **5**−**7** exist in keto/enol equilibria in $CDCl_3$ solution, as indicated by their ^{1}H and ^{13}C NMR spectra (see Exp. Sect.). The 3-substituted *N*-methyltetramic acids **8**−**10** exist in their enol forms in $CDCl_3$ solution, as there were no signals attributable to methine protons in their ^{1}H NMR spectra, whereas their ^{13}C NMR spectra lacked any resonance characteristic of the sp^3-CH methine group expected for the keto tautomers.

Computational Details

We performed ab initio calculations by use of the B3LYP[16] hybrid density functional method with the aid of NWChem 4.1 Computational Chemistry software.[17] Equilibrium and transition state structures were optimised with the pVDZ basis set, which provides highly accurate structures[18] and at the same time is affordable in terms of computational cost. Frequency calculations were performed at the optimised geometries in order to verify that they were indeed equilibrium states (minima) or transition states (saddle points). Energetics were obtained from single-point calculations at the optimised structures with the larger pTZV basis set designed to improve the energies obtained with the pVDZ basis.[19] It was not necessary to augment the pTZV basis with extra diffuse functions, as tests we carried out (augmentation with "Pople-style" diffuse functions:[20] s on hydrogen, s and p on other elements) resulted in changes in the relative energies of our anionic species of less than 1.0 kJ/mol while making our calculations considerably more expensive. Solvation effects in THF (dielectric constant $\varepsilon = 7.52$) and in water (dielectric constant $\varepsilon = 78.4$) were estimated by single-point energy calculations at the optimised geometries with the COSMO model.[21]

Results Relating to the Cyclisation Mechanism Obtained by the B3LYP Hybrid Density Functional Method

We selected methyl 4-amino-3-hydroxy-2-(methoxycarbonyl)-2-butenoate (**I**, Scheme 7), the simplest molecule of the class we are interested in, as a model for a detailed study of the cyclisation reaction. From our calculations, the 3-hydroxy-2-butenoate tautomeric form is the most stable of the possible tautomeric forms for these compounds. The same is true for the resulting tetramic acids, which therefore appear as 3-substituted 4-hydroxypyrrolin-2-ones throughout the text.



I R = H
IV R = CH_3

I′

III R = H
VI R = CH_3

Scheme 7

The cyclisation of **I** by intramolecular nucleophilic attack of the nitrogen lone pair on the carbonyl group would produce structures such as **I′**. We found that structures of this type are not stable neither as equilibrium states nor as transition states, and attempts to optimize them led back to the reactant molecule **I**, a strong indication that this form of cyclisation involving the neutral molecule **I** is not favoured.

In the Exp. Sect. of this paper it is mentioned that the cyclisation reaction takes place in a solution of NaH in THF when the *N*-substituents are a Boc group and an H atom. If the *N*-substituents are a Boc group and a methyl group, no cyclisation takes place. Only after the Boc group is replaced by an H atom can cyclisation occur, again under highly basic conditions, this time brought about by use of a solution of MeONa in MeOH. These facts suggest that the presence of at least one proton attached to the nitrogen atom is a necessary condition for the intramolecular cyclisation reaction. It is therefore likely that the cyclisation is initiated by the abstraction of this proton. The deprotonation reaction requires some other molecule such as a

basic anion to bind the departing proton. In the case of MeONa in methanol solution, a methoxide anion would be the proton receptor. The reactant structure corresponding to this situation is the structure **I−OMe** shown in Figure 1, in which a methoxide anion is hydrogen-bonded to one of the protons of the nitrogen atom of **I**. Starting from the **I−OMe** structure, we were able to locate the transition state **I−OMe-TS** corresponding to the process of proton transfer from the nitrogen atom to the oxygen atom of the methoxide anion. The optimized geometry of this transition state structure is shown in Figure 1. On the product side we obtained structure **II-HOMe**, the outcome of the deprotonation reaction, also shown in Figure 1.

The sequence of structures **I−OMe**, **I−OMe-TS** and **II-HOMe** shows that the deprotonation reaction is followed by an intramolecular nucleophilic attack of the nitrogen lone pair on the carbonyl carbon atom, which results in the formation of the five-membered heterocyclic ring present in the **II-HOMe** structure. In summary, the reaction consists of two stages: (1) proton transfer from the nitrogen atom

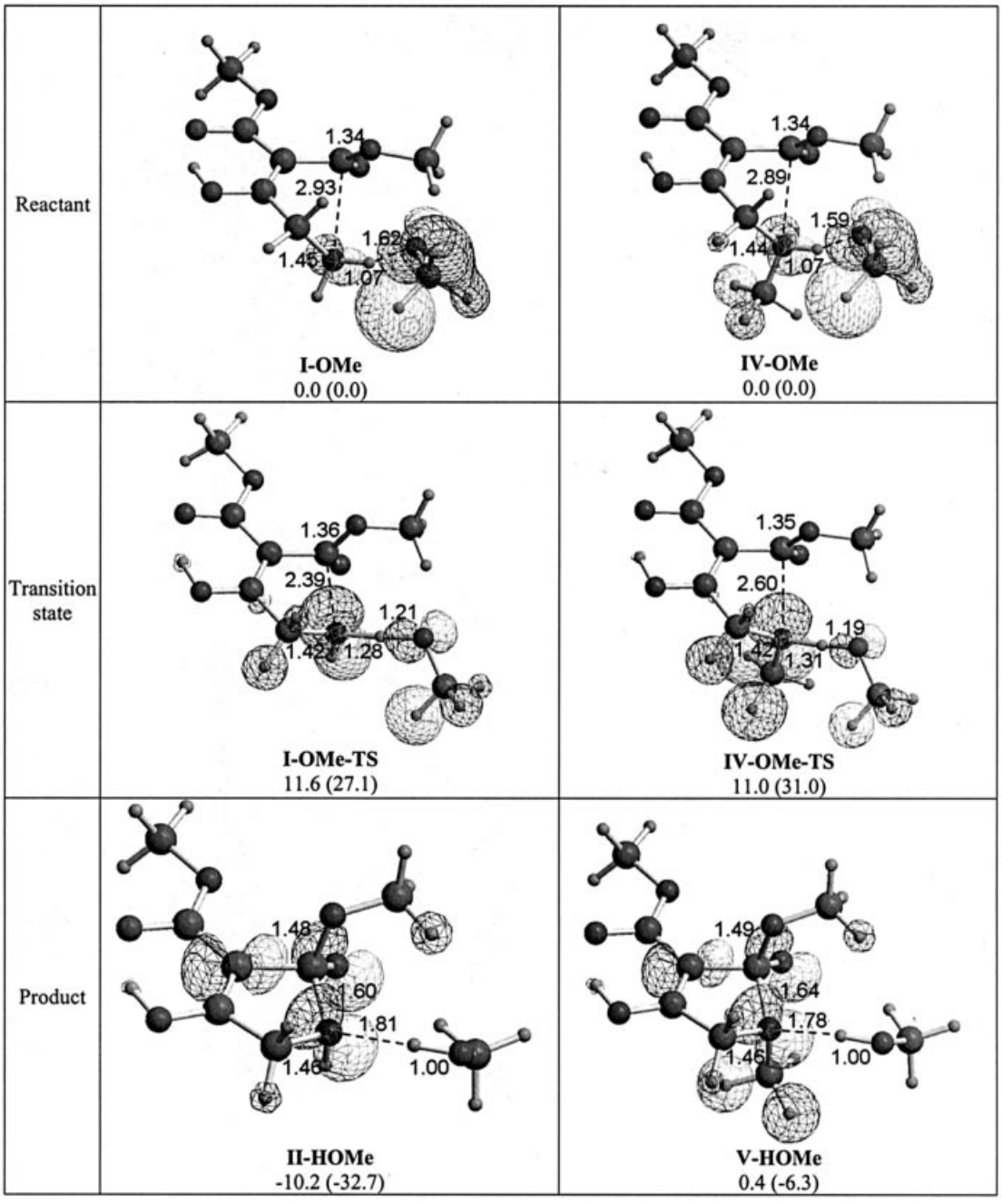


Figure 1. Deprotonation and cyclisation of **I** and **IV**; a methoxide anion acts as the receiver of one of the protons initially attached to the nitrogen atom; the optimized structures of the reactant, transition state and products are shown, including isosurface plots of their highest occupied molecular orbital (HOMO) at 0.018 a.u.; the relative energy in kJ/mol of each structure in vacuo and in THF solvent (in parentheses) is shown under its name; selected distances in Å are also shown

to the methoxide ion oxygen atom (structure **I−OMe-TS**), and (2) ring closure through formation of a carbon−nitrogen bond (structure **II-HOMe**). Figure 1 also shows the highest occupied molecular orbital (HOMO) of each structure as an isosurface plot. Initially the HOMO is almost entirely localised on the methoxide anion, which bears the negative charge. As the reaction proceeds, the HOMO vanishes from the methoxide anion and appears along the path of the nucleophilic attack, so that the formation of the C−N bond is concerted with the proton abstraction. The energy is increased by 11.6 kJ/mol (27.1 kJ/mol in THF solvent) upon reaching the transition state and decreased by 21.8 kJ/mol (59.8 kJ/mol in THF solvent) when moving from the transition state to the products.

The above cyclisation mechanism for the model molecule **I** is also valid for its *N*-methyl analogue **IV** (Scheme 7). Structures **IV−OMe**, **IV-OMe-TS** and **V-HOMe** are obtained (Figure 1), closely resembling structures **I-OMe**, **I-OMe-TS** and **II-HOMe** in their geometrical parameters, the main difference being that they each contain a methyl group attached to the nitrogen atom. On going from the reactant **IV-OMe** to the transition state **IV-OMe-TS**, the energy is increased by 11.0 kJ/mol (31.0 kJ/mol in THF solvent). It is then decreased by 10.6 kJ/mol (37.3 kJ/mol in THF solvent) upon going from **IV-OMe-TS** to the product **V-HOMe**. The fact that less energy is released in the cyclisation reaction of **IV** than in the case of **I** can be attributed to steric effects caused by the extra methyl group of **IV**.

The THF solvent affects the energetics of the cyclisation of **I-OMe** and **IV-OMe** by stabilizing the equilibrium states − and especially the products in the case of **IV-OMe** − more than the transition states. This results in a more exothermic reaction overall, albeit with a higher energy barrier.

Structures **II-HOMe** and **V-HOMe** are stable intermediates that will result in the formation of the desired tetramic acids **III** and **VI**, respectively. This will require the expulsion of one methoxy unit and the attainment of a planar geometry by the atoms participating in the ring, as shown in the optimised structures in Figure 2. We must also note that the methanol molecule that was hydrogen-bonded to the nitrogen atom is also gone, since the nitrogen atom now has a planar geometry.

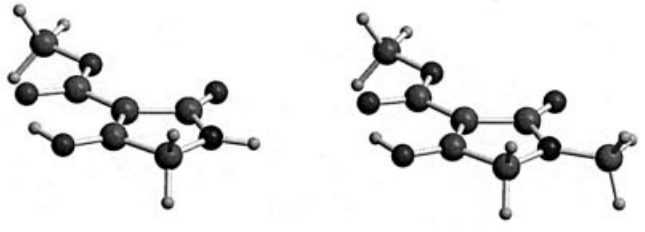


Figure 2. Left: optimized structure of **III**; right: optimized structure of **VI**; note that the nitrogen atom has a completely planar geometry in both cases

We were not able to locate transition states for this last stage in the formation of the tetramic acids. This could occur either by dissociation of **II-HOMe**, still under basic conditions, in which case the energy we obtain is 137.2 kJ/mol in vacuo and −10.9 kJ/mol in THF, or during the aqueous workup with the participation of an oxonium ion H_3O^+:

II-HOMe → **III** + OMe^- + HOMe

II-HOMe + H_3O^+ → **III** + 2 HOMe + H_2O

The energy we obtain for this reaction with H_3O^+ is −785.9 kJ/mol in vacuo and −250.9 kJ/mol in water. Similarly, for **IV-HOMe** dissociation under basic conditions we obtain an energy of 118.1 kJ/mol in vacuo and −19.5 kJ/mol in THF, while under the acidic conditions of the aqueous workup we find an energy of −805.0 kJ/mol in vacuo and −257.7 kJ/mol in water. Here the solvent also plays an important role, particularly in the case of the dissociation reactions of **II-HOMe** and **IV-HOMe** under basic conditions, which are favoured energetically when the THF solvent is taken into account.

Conclusions

We have synthesized novel statine analogues, namely 2-substituted 4-[Boc(methyl)amino]-3-hydroxy-2-butenoates, through *C*-acylation reactions between active methylene compounds and the *N*-hydroxysuccinimidyl ester of Boc-sarcosine. The NMR spectra of these compounds are rather complicated because of the existence of two rotamers arising from hindered rotation around the C−N bond and of the two possible tautomeric forms (keto and enol) in $CDCl_3$ solution.

The new compounds were used as precursors for the synthesis of 3-substituted *N*-methyltetramic acids. After removal of the Boc protecting groups, the isolated hydrochloride salts were easily cyclised to the desired heterocyclic compounds. This new approach to the synthesis of *N*-methyltetramic acids provides a convenient and versatile way to these biologically active species.

Moreover, the mechanism of the cyclisation reaction of 4-amino-2-butenoates was studied theoretically by the B3LYP hybrid density functional method in vacuo and in solvent environments. We suggest that a plausible cyclisation path would involve the formation of an anion by proton abstraction from the nitrogen atom. The presence of at least one hydrogen atom attached to the nitrogen atom is therefore necessary for cyclisation.

Experimental Section

General: Melting points were determined with a Gallenkamp MFB-595 melting point apparatus; the results are given without correction. The FT IR spectra were recorded with a Nicolet Magna IR 560 instrument. The ^1H and ^{13}C NMR spectra were recorded with a Varian Gemini 2000 spectrometer. The chemical shifts are given relative to TMS; *J* values are given in Hz. TLC analyses: Silica gel Fluka F254 plates (0.2 mm layer thickness). Flash column chromatography: Merck Kieselgel 60 (230−400 mesh). Elemental

analyses were obtained with a EuroVector EURO EA Elemental Analyser.

***N*-Hydroxysuccinimidyl Ester of *N*-Boc-sarcosine (1):** The ester was prepared as described in ref.[13]

General Procedure for the *C*-Acylation of Active Methylene Compounds with the *N*-Hydroxysuccinimidyl Ester of *N*-Boc-sarcosine (1): The active methylene compound (10 mmol) was added dropwise at 0 °C to a mixture of sodium hydride (0.17 g, 7 mmol, 55−60% sodium hydride in oil) in anhydrous THF (20 mL) and the resulting mixture was stirred at room temperature for 1 h. The *N*-hydroxysuccinimidyl ester of *N*-(*tert*-butoxycarbonyl)sarcosine (1 g, 3.5 mmol) was then added, and stirring was continued at room temperature for 1 h. The solvent was evaporated in vacuo and the residue was diluted with water. The aqueous layer was washed once with diethyl ether, acidified with 10% hydrochloric acid and extracted with CH_2Cl_2. The organic layer was separated and dried (Na_2SO_4), and the solvents were evaporated in vacuo. The oily residue was purified by flash column chromatography (petroleum ether/EtOAc, 8:2) to afford the corresponding 2-substituted 4-[Boc-(methyl)amino]-3-hydroxy-2-butenoates **2**−**5** as colourless, viscous oils.

Methyl 4-[(*tert*-Butoxycarbonyl)(methyl)amino]-3-hydroxy-2-methoxycarbonyl-2-butenoate (2): Yield 0.81 g, 76%; TLC (petroleum ether/EtOAc, 8:2) R_f = 0.37. $C_{13}H_{21}NO_7$ (303.3): calcd. C 51.48, H 6.98, N 4.62; found C 51.31, H 7.30, N 4.60. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.40 and 1.44 [2 s, each 4.5 H, $C(CH_3)_3$], 2.85, 2.86, 2.89 and 2.91 (4 br. s, 3 H, NCH_3), 3.73, 3.75, 3.79 and 3.82 [4 s, 6 H, $(COOCH_3)_2$], 4.12, 4.18, 4.26 and 4.34 (4 br. s, 2 H, 4-H), 4.58 (s, 0.5 H, 2-H, keto form), 14.03 (br. s, 0.6 H, OH, enol form) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.9 and 28.1 [$(CH_3)_3$], 35.4 and 35.5 (N−CH_3), 52.0, 52.5 and 53.2 (COO*C*H$_3$), 57.4 and 58.1 (C-4), 61.8 and 62.2 (C-2, keto form), 80.2, 80.5 and 80.8 [$(CH_3)_3$*C*], 98.1 and 98.5 (C-2, enol form), 155.4 and 155.7 [$(CH_3)_3$CO*C*O], 164.7, 164.8 and 167.1 (C-1, enol form), 172.0 (C-1, keto form), 181.6 and 182.0 (C-3, enol form), 195.6 and 195.7 (C-3, keto form) ppm. IR (CH_2Cl_2): $\tilde{\nu}$ = 3055 cm^{-1} m, 2983 m (OH), 1736 s (C=O, β-oxo ester, keto form), 1698 s (C=O, β-oxo ester, enol form and C=O, urethane).

Ethyl 4-[(*tert*-Butoxycarbonyl)(methyl)amino]-2-(ethoxycarbonyl)-3-hydroxy-2-butenoate (3): Yield 0.93 g, 81%; TLC (petroleum ether/EtOAc, 8:2). R_f = 0.60. $C_{15}H_{25}NO_7$ (331.4): calcd. C 54.37, H 7.60, N 4.23; found C 54.10, H 8.05, N 4.61. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29 [t, *J* = 7.2 Hz, 6 H, $(COOCH_2CH_3)_2$], 1.42 and 1.46 (2 s, each 4.5 H, $C(CH_3)_3$], 2.86, 2.88, 2.91 and 2.93 (4 br. s, 3 H, NCH_3), 4.13 (s, 1 H, 4-H), 4.19−4.35 [m, 5 H, $(COOCH_2CH_3)_2$ and 4-H], 4.55 (s, 0.5 H, 2-H, keto form), 13.97 and 14.01 (2 br. s, each 0.2 H, OH enol form) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.8 and 13.9 ($COOCH_2CH_3$), 28.0 and 28.2 [$(CH_3)_3$], 35.4 and 35.5 (NCH_3), 57.4 and 58.2 (C-4), 60.9, 61.5 and 61.7 (C-2, keto form), 62.3, 62.5 and 62.8 (COO*C*H$_2$CH$_3$), 80.2, 80.5 and 80.8 [$(CH_3)_3$*C*], 98.8 and 98.3 (C-2, enol form), 155.5 and 156.2 [$(CH_3)_3$CO*C*O], 164.5, 165.5 and 166.8 (C-1, enol form), 171.6 (C-1, keto form), 181.2 (C-3, enol form), 195.9 (C-3, keto form) ppm. IR (CH_2Cl_2): $\tilde{\nu}$ = 2982 cm^{-1} m (OH), 1731 s (C=O, β-oxo ester, keto form), 1693 s (C=O, β-oxo ester, enol form and C=O, urethane).

Ethyl 4-[(*tert*-Butoxycarbonyl)(methyl)amino]-3-hydroxy-2-(methylsulfonyl)-2-butenoate (4): Yield 0.80 g, 68%; TLC (petroleum ether/EtOAc, 8:2). R_f = 0.51. $C_{13}H_{23}NO_7S$ (337.4): calcd. C 46.29, H 6.87, N 4.15; found C 45.97, H 7.21, N 4.50. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29 [t, *J* = 6.9 Hz, 6 H, $(COOCH_2CH_3)_2$], 1.38 and 1.43 (2 s, each 4.5 H, $C(CH_3)_3$], 2.86 (s, 3 H, NCH_3), 3.19 (s, 3 H, SO_2CH_3), 4.22−4.29 (m, 4 H, $COOCH_2CH_3$ and 4-H), 4.98 and 5.02 (2 br. s, 1 H, 2-H, keto form) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.67 and 13.74 ($COOCH_2CH_3$), 27.95 and 28.06 [$(CH_3)_3$], 35.2 and 35.5 (NCH_3), 41.5 (SO_2CH_3), 59.1 and 59.9 (C-4), 62.6 ($COOCH_2CH_3$), 63.3 and 63.4 (C-2, keto form), 80.7 and 80.8 [$(CH_3)_3$*C*], 155.2 and 156.1 [$(CH_3)_3$CO*C*O], 163.2 (C-1, keto form), 193.0 and 193.2 (C-3, keto form) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 3019 cm^{-1} s (OH), 1737 m (C=O, β-oxo ester, keto form), 1691 m (C=O, β-oxo ester, enol form and C=O, urethane), 1328 s (SO_2).

General Procedure for the Removal of the *tert*-Butoxycarbonyl Protecting Group: 2-Substituted 4-[Boc(methyl)amino]-3-hydroxy-2-butenoate (3 mmol) was dissolved in 1 mL of a saturated solution of HCl in MeOH (for compound **2**) or EtOH (for compounds **3** and **4**) and the mixture was stirred at room temperature for 0.5 h (monitored by TLC). The solvent was evaporated and the residue was triturated with diethyl ether to give the corresponding hydrochloride salt as a highly hygroscopic white solid.

Methyl 3-Hydroxy-2-(methoxycarbonyl)-4-(methylamino)-2-butenoate Hydrochloride (5): Yield 0.70 g, 98%. $C_8H_{14}ClNO_5$ (239.6): calcd. C 40.43, H 5.09, N 5.89; found C 40.65, H 5.30, N 5.64. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.78,2,79 and 2.81 (3 br. s, 3 H, NCH_3), 3.73, 3.77, 3.81 and 3.86 [3 s, 6 H, $(COOCH_3)_2$], 4.27 (pseudo-triplet, 1 H, 4-H), 4.42 (pseudo-triplet, 1 H, 4-H), 4.93 (s, 0.5 H, 2-H, keto form), 9.55 (br. s, 1 H, NH), 9.74 (br. s, 0.9 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 33.1 and 33.2 (N-CH_3), 52.5, 52.7, 53.2 and 53.7 (COO*C*H$_3$), 49.3 (C-4), 63.0 (C-2, keto form), 100.2 (C-2, enol form), 164.2 and 164.8 (C-1, enol form), 171.9 (C-1, keto form), 175.0 (C-3, enol form), 192.6 (C-3, keto form) ppm. IR (CH_2Cl_2) : $\tilde{\nu}$ = 3054 cm^{-1} m and 2986 m (OH), 2685 br. m (NH_2^+), 1736 m (C=O, β-oxo ester, keto form), 1701 m (C=O, β-oxo ester, enol form).

Ethyl 2-(Ethoxycarbonyl)-3-hydroxy-4-(methylamino)-2-butenoate Hydrochloride (6): Yield 0.76 g, 95%. $C_{10}H_{18}ClNO_5$ (267.7): calcd. C 44.86, H 6.77, N 5.23; found C 44.49, H 7.15, N 4.94. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27−1.35 [m, 6 H, $(COOCH_2CH_3)_2$], 2.79 (s, 3 H, NCH_3), 4.20−4.38 [m, 6 H, $(COOCH_2CH_3)_2$ and NCH_2), 4.75 (s, 0.5 H, 2-H, keto form), 9.80 and 9.90 (2 br. s, 2 H, NH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 13.8 and 13.9 ($COOCH_2CH_3$), 33.0 and 33.1 (N-CH_3), 49.1 (C-4), 61.4 and 62.5 ($COOCH_2CH_3$), 63.0 and 63.6 (C-2, keto form), 100.9 (C-2, enol form), 163.7 and 164.6 (C-1, enol form), 171.4 (C-1, keto form), 174.2 (C-3, enol form), 192.4 (C-3, keto form) ppm. IR (CH_2Cl_2: $\tilde{\nu}$ = 3052 cm^{-1} m and 2976 m (OH), 2931 and 2870 br. m (NH_2^+), 1733 s (C=O, β-oxo ester, keto form), 1695 m (C=O, β-oxo ester, enol form).

Ethyl 3-Hydroxy-4-(methylamino)-2-(methylsulfonyl)-2-butenoate Hydrochloride (7): Yield 0.79 g, 97%. $C_8H_{16}ClNO_5S$ (273.7): calcd. C 35.10, H 5.89, N 5.12; found C 35.00, H 5.85, N 4.99. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ = 1.20−1.27 [m, $(COOCH_2CH_3)_2$], 2.64 and 2.66 (2 br. s, 3 H, NCH_3), 3.16 (br. s, 3 H, SO_2CH_3), 4.15−4.27 [m, 5 H, $(COOCH_2CH_3)_2$, 4-H and 2-H, keto form) ppm. IR (KBr): $\tilde{\nu}$ = 2937 cm^{-1} s, 2758 s, 2693 s (NH and OH), 1749 s (C=O, β-oxo ester, keto form), 1341 s (SO_2).

General Procedure for the Synthesis of 3-Substituted *N*-Methyltetramic Acids: The hydrochloride salt (2 mmol) was added to a solution of sodium alkoxide in alcohol (MeONa in dry MeOH for compound **5** and EtONa in EtOH for compounds **6** and **7**) prepared from sodium (4 mmol) in alcohol (1 mL). The reaction mixture was stirred at room temperature for 24 h (monitored by TLC). The solvent was evaporated in vacuo and the solid residue was dissolved in water. The aqueous solution was acidified with 10% hydrochloric

acid and extracted with CH_2Cl_2. The organic layer was separated and dried (Na_2SO_4), and the solvents were evaporated in vacuo to afford the corresponding tetramic acids as solids.

3-(Methoxycarbonyl)-1-methyltetramic Acid (8): White solid. Yield 0.30 g, 89%; m.p.188−191 °C. $C_7H_9NO_4$ (171.05): calcd. C 49.12, H 5.30, N 8.18; found C 49.48, H 5.67, N 7.94. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.98 (s, 3 H, NCH_3), 3.91 (s, 3 H, $COOCH_3$), 3.97 (s, 2 H, 5-H) ppm. IR (KBr): $\tilde{\nu}$ = 2952 cm^{-1} m br. (OH), 1865 m, 1714 s, 1600 s (C=O).

3-(Ethoxycarbonyl)-1-methyltetramic Acid (9): White solid. Yield 0.35 g, 94%; m.p. 183−186 °C (ref.[22] 190−192°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (t, J = 7.2 Hz, 3 H, $COOCH_2CH_3$), 2.97 (s, 3 H, NCH_3), 3.96 (s, 2 H, 5-H), 4.39 (q, J = 7.2 Hz, 2 H, $COOCH_2CH_3$) ppm. IR (KBr): $\tilde{\nu}$ = 2982 cm^{-1} m br. (OH), 1840 m, 1704 s, 1607 s (C=O).

1-Methyl-3-methylsulfonyltetramic Acid (10): Off-white solid. Yield 0.29 g, 77%; m.p. 164−166 °C (from CH_2Cl_2/petroleum ether). $C_6H_9NO_4S$ (191.02): calcd. C 37.69, H 4.74, N 7.32; found C 37.78, H 4.97, N 7.12. ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): δ = 2.83 (s, 3 H, NCH_3), 3.11 (s, 3 H, SO_2CH_3), 3.92 (s, 2 H, 5-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$): δ = 28.4 (NCH_3), 42.8 (SO_2CH_3), 52.1 (C-5), 105.5 (C-3), 165.7 (C-2), 175.6 (C-4) ppm. IR (KBr): $\tilde{\nu}$ = 2928 cm^{-1} m br. (OH), 1596 s (C=O), 1302 s, 1136 s (SO_2).

Supporting Information (see also the footnote on the first page of this article): Cartesian coordinates of optimised structures, energies of single-point calculations.

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