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Acontyl-derived polymers for biomedical applications. Modeling study of cis–trans isomerisation

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Abstract. Soluble polymers have been prepared that are designed to undergo enhanced rates of hydrolysis at pH values less than that observed in blood circulation. The degradable element in the polymer mainchain is derived from *cis*-aconityl acid and is defined by a carboxylic acid pendent functionality (C-4) that is cis across a double bond to an amide at C-1 in the polymer mainchain. While degradation studies in vitro have confirmed these polymers do undergo enhanced rates of degradation at acidic pH values, there is also increasing evidence that during the degradation process the double bond isomerises to the trans configuration and thus prevents the full degradation of a polymer. From a molecular modelling perspective we are seeking to understand the propensity for this cis–trans isomerisation and the mechanism of this cis–trans isomerisation is discussed.

Keywords: Ab initio – Molecular modeling – Hydrolytically degradable – Polymer – Aconityl acid

1 Introduction

Conjugation of bioactive moieties (e.g. conventional drugs and proteins) to soluble macromolecules (e.g. polymers and proteins) is an active area of therapeutic development. In the case of water-soluble polymers there are inherent properties that can be imparted to conjugated bioactive molecules. These properties include prolonging blood circulation time, minimising toxic side effects, exploiting permeability gradients in tissue and favourably altering biodistribution to increase efficacy. Additionally soluble polymers are being developed that are inherently therapeutic owing to their polyvalent interactions [1]. Considerable work has been done to examine polymers that do not readily degrade in the body. However to consider treating chronic conditions where repeat administration of a therapeutic conjugate would be necessary, it is imperative that degradable polymers be developed.

We are developing water-soluble polymers that will hydrolytically degrade at weakly acid pH values [2]. The rate and mode of degradation will depend on whether uptake into cells occurs and whether the polymer encounters tissue sites that display lowered pH environments (e.g. tumours, sites of infection and gastrointestinal tract). Such hydrolytically labile polymers must be stable in solid form and predominantly stable in solution at neutral pH while undergoing enhanced degradation at acidic pH values.

To aid polymer development, degradable elements are first being prepared and characterised, then incorporated into the polymer mainchain. An example of such a degradable element is derived from aconityl acid [2], which is a natural metabolite. The aconityl derived soluble polymer **1** has a carboxylic acid (C-4) cis to a hydrolytic bond (C-1). Such polymers have a pH-dependent degradation profile with higher rates of hydrolysis at acidic pH values owing to intramolecular-assisted C-4 acid-catalysed hydrolysis at the C-1 bond. The enforced propinquity of the pendent carboxylic acid at C-4 is necessary to ensure that intramolecular interactions facilitate enhanced rates of hydrolysis at the C-1 amide. Evaluation of the in vitro biocompatibility indicated this first polymer and its degradation products were not cytotoxic to B16F10 cells or caused red blood cell lysis. However, the degradation profile of this first-generation polymer indicated there was incomplete degradation of each aconityl unit in the polymer mainchain.

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Polymer **1** was derived from *cis*-aconityl anhydride **2**. This commercially available compound has been used for the conjugation of drugs and proteins through the reaction of an aliphatic amino moiety with the anhydride at C-1 [3]. The synthesis of polymer **1** was also reliant on the reaction of an aliphatic amine at C-1. Unfortunately a significant number of competitive side reactions occur leading to decarboxylation at C-6, double bond isomerisation and decomposition.

These competitive reactions resulted in the incorporation nonhydrolytically labile elements in the polymer mainchain. Additionally, this initial research demonstrated that the C-2, 3 double bond can undergo facile isomerisation to C-3,5 and in the case of *cis*-aconityl acid **5** (Fig. 1), trans isomerisation also occurs. It was possible by interfacial reaction of aconityl anhydride **2** and glycine to prepare the aconityl analogue **3** in good yield and purity [4].

We have previously shown by modelling studies that the presence of a long poly(ethylene glycol) chain in the first-generation polymer **1** brings in close proximity the OH of the C-4 carboxylic group to the nitrogen atom N-1 and can affect the electron density of molecular orbitals with consequent contribution to the reaction rate associated with hydrolysis. This initial study indicated that the structural features of the aconityl motif in the polymer mainchain can influence degradation [5]. In this current modelling study, we primarily examine the propensity for double-bond isomerisation in the polymer mainchain to determine what structural features at C-2 will stabilise the polymer structure by minimising double-bond isomerisation and possibly to allow for cleaner reaction with the parent aconityl anhydride **2**.

Materials and methods

Computational methods

The modelling work presented here was performed using Chem2-Pac [6], MOPAC [7], Gamess US [8], Molden [9] and Molekel [10] software packages. In order to model the degradable motif and reaction, two different levels of theory were used (semiempirical and ab initio). The results of each level were used as input to the next higher level of modelling. The structures of each monomer derivative were initially optimised by MOPAC 6.0 in the CHEM2PAC environment. The method used was PM3. Semiempirical PM3 geometries were initially obtained and used to acquire HF/6-31G(*) optimised geometries, except for the largest unit that was optimised using an HF/3-21G(*) basis set. Keeping these geometries, we thereafter obtained single-point energies at higher levels of theory, namely, HF/6-311(*).

Prediction of the reaction pathway was based on the energy levels of the reactants and products, and the activation energies, ΔE_A. Activation energies were predicted as the difference between the energies of reactant and transition states. Transition states for the possible reactions and isomerisation pathways were calculated by MOPAC SADDLE calculations and their energies were again calculated at the HF/6-311G (*) level. All calculations were performed with the Gamess-US and visualized with the Molden 3.7 program packages.

All energies in this work are presented in two different ways: the tables contain energies expressed in hartrees, while the figures present energy values in kilocalories per mole. The HOMO and LUMO energy values are presented in hartrees.

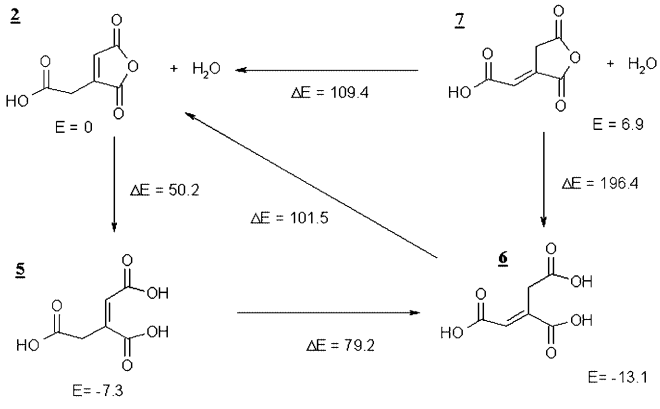


Fig. 1. Possible reaction pathways for isomerisation of the aconityl monomer double bond (*numbers* represent the energies in kcal/mol and relative to the energy of the *cis*-aconityl anhydride isomer **2**)

Results and discussion

Cis–trans isomerisation of aconityl precursors

Enhanced rates of hydrolytic degradation for aconityl-derived polymers occur at acidic pH values. Degradation rates of the polymer mainchain will invariably change owing to the double-bond isomerisation that occurs during synthesis and within the polymer mainchain. Initially, we used ab initio calculations to study the cis–trans isomerisation of the free aconityl acids **5** and **6** and their associated anhydrides (**2** and **7**, respectively in Fig. 1). The anhydride **2** is used in the synthesis of aconityl polymers **1**, but upon hydrolysis may potentially rearrange to anhydride **7** to yield a trans double bond that ends up in the polymer mainchain.

Results of the calculations are summarized in Fig. 1 and in Table 1. The HOMO–LUMO energies are shown in Table 2.

As expected aconityl anhydride **2** displays a lower energy than the trans isomer **7**. HOMO and LUMO orbitals were considered as an indicator of the reactivity and the difference in energy between the HOMO and LUMO levels ($\Delta E_{L-H} = 0.4736$ hartrees) and indicates that *cis*-aconityl anhydride **2** is generally less reactive than **7** ($\Delta E_{L-H} = 0.4589$ hartrees).

Reaction of *cis*-anhydride **2** with water as shown in Fig. 2 to produce *cis*-aconityl acid **5** ($\Delta E_A = 50.2$ kcal/mol) is more favourable than the reaction of the trans anhydride **7** with water ($\Delta E_A = 196.4$ kcal/mol). This is probably due to the HOMO and LUMO orbitals of **2** being localized on the cyclopentyl ring at both polar carbonyl bonds where reaction of an incoming reagent is required (Fig. 3). In constrast, the HOMO of anhydride **7** is exocyclic and the LUMO is only partially localised within the ring at the carbonyl where the reaction is desired.

Interestingly, the ab initio calculations suggest the *cis*-aconityl anhydride **2** is higher in energy than the corresponding free acid **5**. On the basis of such calcu-

Table 1. Total energies of the different forms of the aconityl monomer and some transition states (energy expressed in hartrees)

Derivative	cis isomer	Transition state	cis anhydride + H_2O	Transition state	trans anhydride + H_2O	Transition state	trans isomer
Basic aconityl monomer	−680.0785698430	−679.9869972247	−680.0670034198	−679.8792679049	−680.0559285811	−679.7540792561	−680.0879565090
Aconityl methyl substituted	−719.1242164080	−719.0215436300	−719.1165100687	–	−719.0831786302	–	−719.1167800448
Aconityl ethyl substituted	−758.1655533695	−758.0735617202	−758.1559526625	–	–	–	−758.1649656846
Amino derivative	−926.6569570122	−926.2493538722	−927.3650535292	–	−927.3498888348	–	−926.7183420734
Monomer **3**	−886.9344080861	–	–	–	–	–	−886.9317618882
Methylated monomer **3**	−925.9765187839	–	–	–	–	–	−925.9724931237
Polymer unit	−1102.4387774500	–	–	–	–	–	−1102.4378913247

lations, reaction of *cis*-aconityl anhydride **2** and water is expected to compete with nucleophiles such as amines. Moreover, hydrolysis of **2** does occur in solution and in competition with amines, thus demonstrating the importance of solvent and traces of water in the reaction mixture.

It appears that cis–trans isomerisation has the highest probability of occurring after hydrolysis of *cis*-aconityl anhydride (i.e. **2** → **5** → **6**). Isomerisation of the cis acid **5** to the trans acid **6** (Fig. 4) appears to be the most favourable route for isomerisation with the lowest calculated activation energy of 79.2 kcal/mol (note other values in Fig. 1). The direct isomerisation of **2** to **7** is much less likely (Fig. 5) and it is also less likely than the transformation of **2** into the trans acid **6**.

The HOMO–LUMO difference for the trans acid **6** ($\Delta E_{L-H} = 0.4613$ hartrees) is lower than the corresponding energy of the cis acid **5** ($\Delta E_{L-H} = 0.4937$ hartrees). If polymerisation conditions are employed where the cis acid **5** is used, then isomerisation to give the trans isomer **6** followed by its reaction during polymerisation would result in *trans*-aconityl fragments in the polymer mainchain. We have noted in some polymers that incomplete degradation occurs [2] and this may partially be due to cis–trans isomerisation during polymerisation or during the degradation process rather than in the polymer mainchain during storage. Our calculations suggest that the sequence for the occurrence of the trans double bond during the polymerisation process is

1. Side reaction of *cis*-aconityl anhydride **2** and water to give *cis*-aconityl acid **5**.
2. Isomerisation of *cis*-aconityl acid **5** to *trans*-aconityl acid **6**.
3. Inclusion of the *trans*-aconityl isomer **6** into the polymer.

Double-bond isomerisation stability in model aconityl amides

To determine the propensity for cis–trans double-bond isomerisation within the polymer mainchain a series of calculations were performed on the model monoamides **8** and **9** (Fig. 6) and the diamides **10** and **11** (Fig. 7). The monomethyl amides, *cis*-**8** and t*rans*-**9**, were examined because during synthesis this amide at C-1 is generally the first one made. Isomerisation after amide formation would lead to incorporation of the trans double bond within the polymer mainchain. The trans isomer **9** has significantly lower energy than the cis isomer **8** (Fig. 6); however, these two isomers are comparably reactive (the energy differences between HOMO and LUMO levels were 0.3087 and 0.315 - hartrees, respectively).

To examine more relevant diamides, ethylene glycol units were incorporated in *cis*-**10** and *trans*-**11** (Fig. 7) to mimic the adjacent covalent bonds near the aconityl fragment within the polymer mainchain. Polymer chain entanglement and repulsion in solution as a function of pH and ionic strength may influence the stability of the

Table 2. Energies of the HOMO and LUMO levels of different forms of the aconityl monomer transition states (energy expressed in hartrees)

	HOMO E_H	LUMO E_L	Difference ΔE_{L-H}
cis-Aconityl acid **5**	−0.40310	0.09060	0.4937
cis-Aconityl anhydride **2**	−0.43360	0.04000	0.4736
trans-Aconityl anhydride **7**	−0.43320	0.02570	0.4589
trans-Aconityl acid **6**	−0.40700	0.05430	0.4613
Methyl-substituted *cis*-aconityl acid **14**	−0.39080	0.10210	0.4929
Methyl-substituted *cis*-aconityl anhydride **12**	−0.481810	0.04390	0.5257
Methyl-substituted *trans*-aconityl anhydride **13**	−0.42910	0.02600	0.4551
Methyl-substituted *trans*-aconityl acid **15**	−0.40240	0.09240	0.4948
Ethyl-substituted *cis*-aconityl acid	−0.38920	0.09800	0.4872
Ethyl-substituted *cis*-aconityl anhydride	−0.40050	0.09030	0.4908
Ethyl-substituted *trans*-aconityl anhydride	−0.40370	0.07030	0.474
Ethyl-substituted *trans*-aconityl acid	−0.40090	0.05300	0.4539
cis amino derivate **8**	−0.47630	−0.16760	0.3087
trans amino derivate **9**	−0.47530	−0.16030	0.315
cis monomer **3**	−0.39970	0.06620	0.4659
trans monomer **16**	−0.39990	0.07690	0.4768
Methylated cis monomer **17**	−0.38710	0.10010	0.4872
Methylated trans monomer **18**	−0.39770	0.08090	0.4786
cis polymer unit **10**	−0.38150	0.07770	0.4592
trans polymer unit **11**	−0.37590	0.06150	0.4374

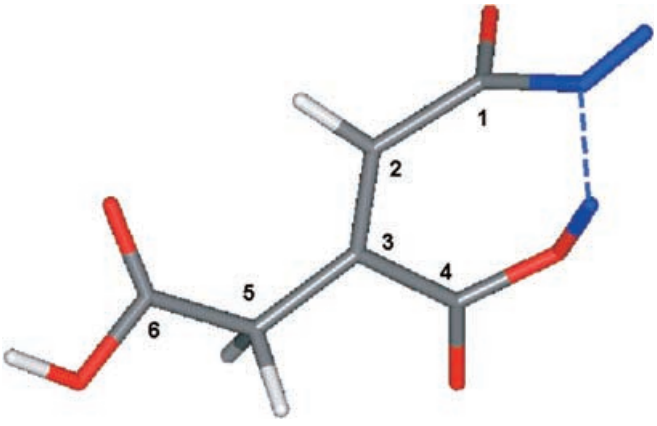


Fig. 2. Transition state of the reaction between **2** and water (water molecule is shown in *blue*)

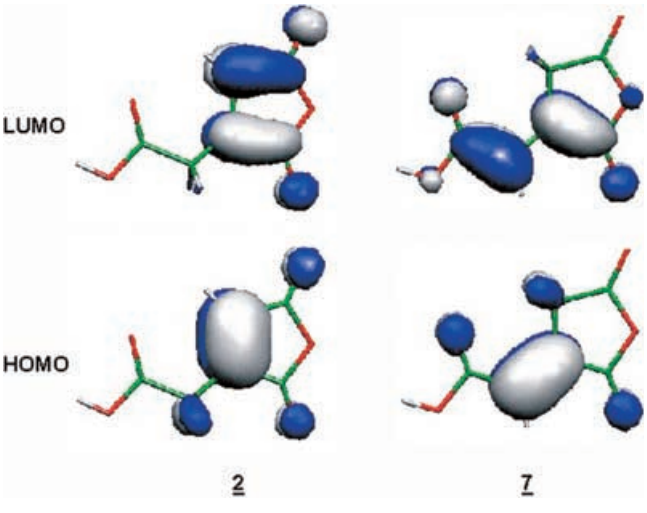


Fig. 3. Optimized geometries and spatial distribution of HOMO and LUMO for the **2** and **7** aconityl anhydrides

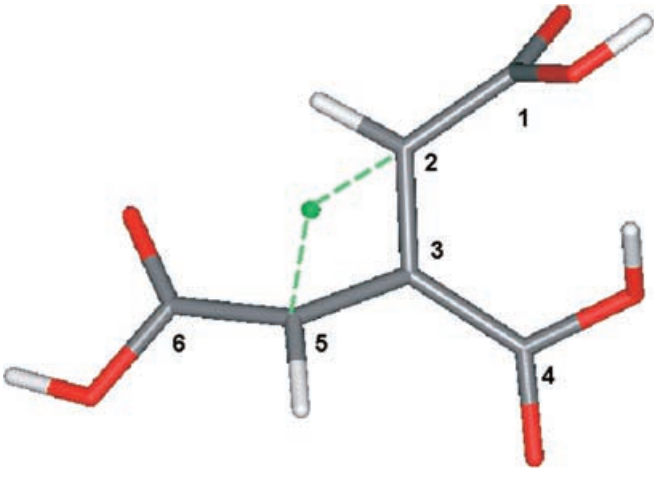


Fig. 4. Transition state of the cis–trans isomerisation of aconityl acid (**5** into **6**)

double bond. Our intent though is to develop soluble polymers that will undergo hydrolysis that is dependent primarily on the structural characteristics of the degradable element (i.e. aconityl motif) rather than differences in polymer properties. In contrast to the monoamides **8** and **9**, the energies of the diamides are much closer together and in fact, the cis isomer **10** has marginally lower energy (about 0.5 kcal/mol) than the trans isomer **11** (Fig. 7). This suggests that should it be possible to prepare the polymer without competitive isomerisation occurring, then once the polymer is formed, the cis double bond may not readily isomerise.

We have recently examined how interactions from the C-4 carboxyl with the C-1/N-1 site within the *cis*-aconityl fragment can lead to enhanced degradation [3]. These interactions are depicted in Fig. 8 for the cis isomer **10**. Similar interactions do not appear as

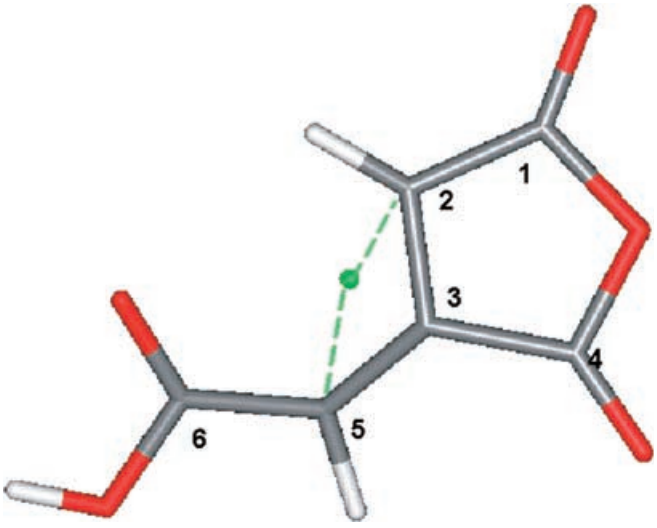


Fig. 5. Transition state of the cis–trans isomerisation of aconityl anhydride (**2** into **7**)

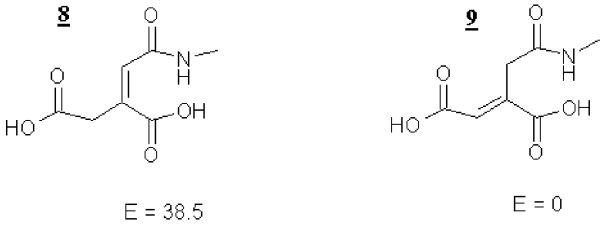


Fig. 6. Aconityl acid derived monomethyl amides, *cis*-**8** and *trans*-**9** with the relative energy difference shown in kcal/mol

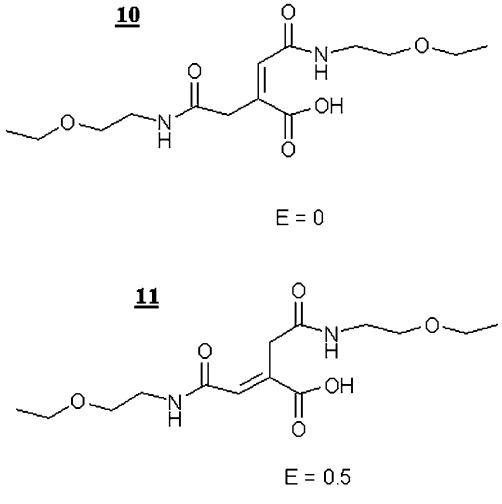


Fig. 7. Aconityl derived diamides *cis*-**10** and *trans*-**11** designed to model the structural features around the double bond within the polymer mainchain. The relative energy difference of the trans isomer **11** to the cis isomer **10** is given in kcal/mol

probable within the trans isomer **11** (Fig. 9). Hydrolytic degradation is known to be faster at lower pH only in the *cis*-aconityl analogues. However, the interactions shown in Fig. 9 do not preclude eventual hydrolysis of polymers possessing trans double bonds.

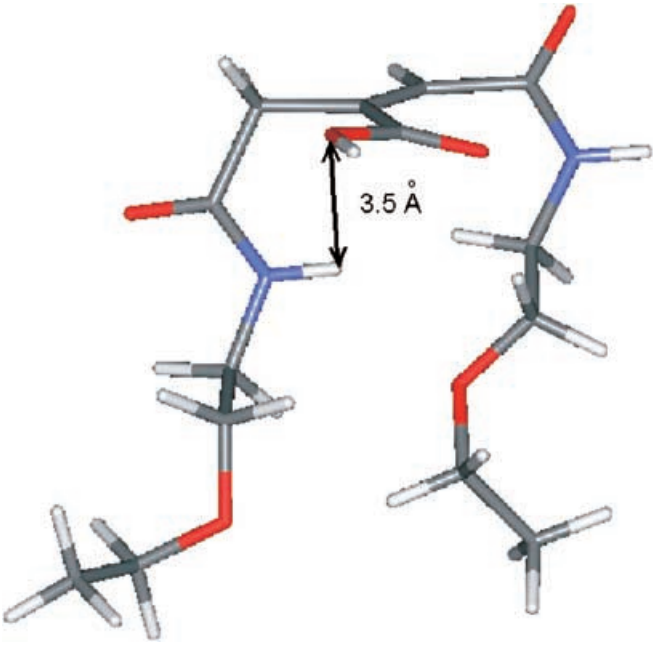


Fig. 8. Optimized geometry of *cis*-**10**. The *arrow* indicates the close proximity of C-1 and N-1 to the C-4 carboxyl OH

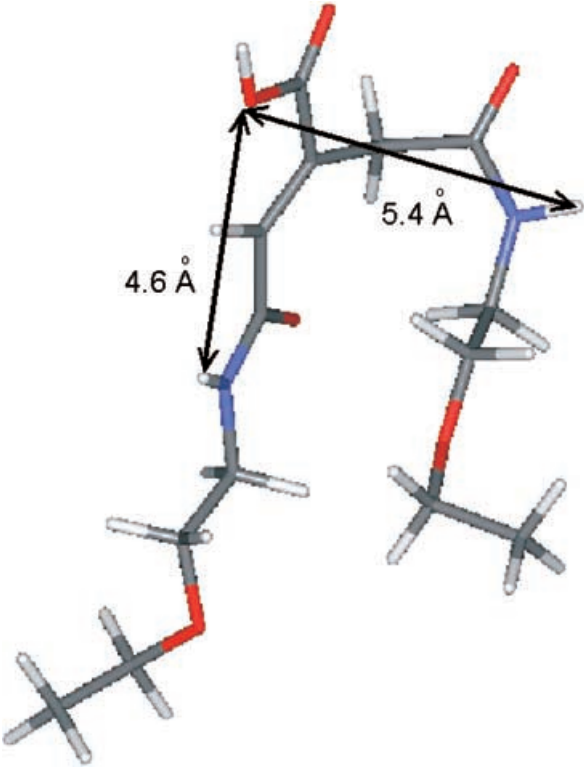


Fig. 9. Optimized geometry of *trans*-**11**. The *arrows* indicate the distance between N-1 and the C-4 carboxyl OH and between N-6 and the C-4 carboxyl OH

Effects of substitution on the C-2 position for double-bond isomerisation

To explore further how double-bond isomerisation could be influenced by simple changes in structure, we

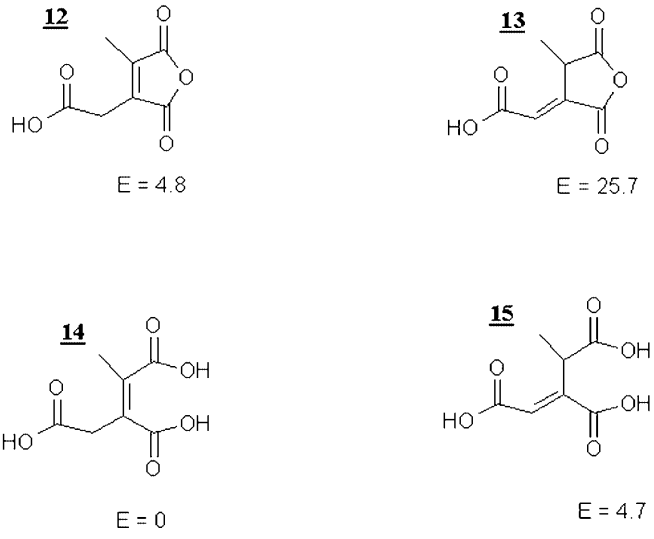


Fig. 10. Methyl substitution at C-2 with energies given in kcal/mol relative to that of the cis isomer **14**

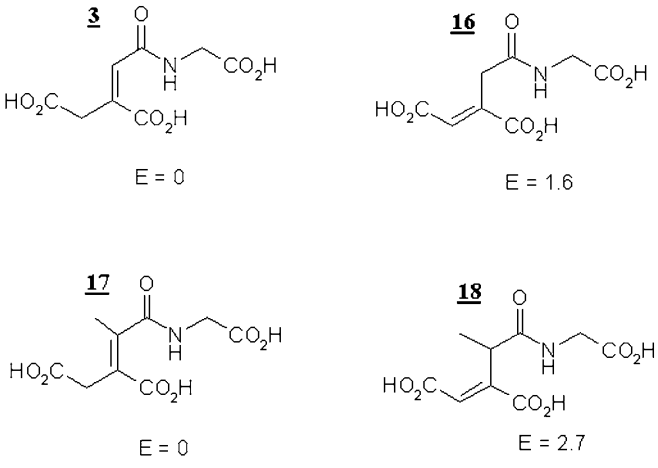


Fig. 11. Derivates of aconityl monomer **3** with the methyl substitution on the position C-2. (*numbers* represent the energies in kcal/mol and relative to the energy of the cis isomer)

examined model structures with alkyl substitution at C-2. The cis double bond between C-2 and C-3 would be tetrasubstituted compared to the trisubstituted trans double bond between C-3 and C-5. The isomeric C-2 methyl-substituted anhydrides **12** and **13** and free aconityl acids **14** and **15** are shown in Fig. 10

Compared to no substitution, methylation at C-2 had a threefold effect on the double-bond isomerisation:

1. The cis acid **14** was the lowest-energy molecule, thus minimising the possibility of the direct isomerisation of **14** into **15** (compare unsubstituted structures in Fig. 1).
2. Increased activation energy for the reaction of **12** → **14** ($\Delta E_A = 64.4$ kcal/mol), therefore decreasing the chances for deleterious side reactions between anhydride and water.
3. Decreased reactivity of the trans acid **15** which, compared to the unsubstituted trans acid **6** (Table 2), would result in a lower chance of its incorporation into the polymer mainchain.

Together these three characteristics of C-2 substitution would make it possible to prepare polymers with a lower incorporation of the *trans*-aconityl fragment. This would ensure a less complicated degradation profile that is derived primarily from the favourable interactions of the cis form.

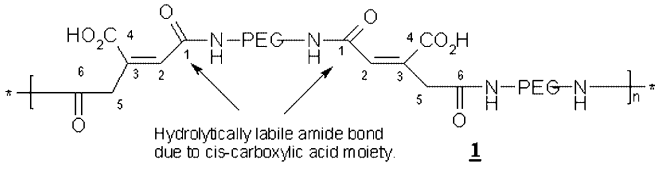
Ethylation of the aconityl anhydride at the C-2 position has only a twofold effect, effects 1 and 2 (some results shown in Table 1); therefore, using longer alkyl chains for the substitution was not considered in our further studies.

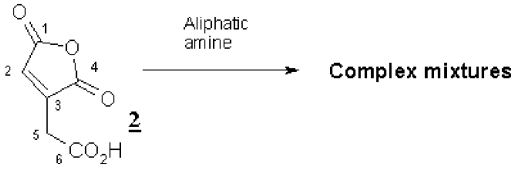
The glycine aconityl analogue **3** has been used as a monomer to prepare aconityl polymers **4**. In contrast to the precursor aconityl acids (*cis*-**5** and *trans*-**6**) the *trans*-glycine adduct **16** is higher in energy than the corresponding *cis*-glycine analogue **3**. Analogous C-2 methylation to give **17** and **18** increases the energy difference between the cis and trans isomers making isomerisation less likely (Fig. 11). Stabilization of the cis monomer is not so significant as in the case of the methylation of the anhydride **2**; therefore, the production of the cis form of **3** would ensure synthesis of the polymer containing only cis double bond, thus having fully degradable product.

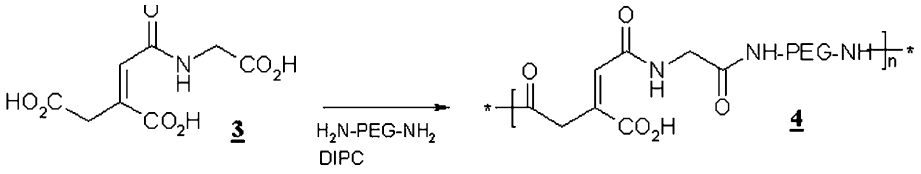
Conclusions

Water-soluble polymers derived from *cis*-aconityl acid **5** capable of enhanced rates of hydrolytic degradation are being developed for biomedical applications. Isomerisation of the *cis* double bond to trans during polymer synthesis, degradation and/or storage is not desired. Isomerisation to the trans isomer is most likely from the free aconityl acid **5** which can be formed in side reactions between *cis*-aconityl anhydride **2** and water during polymer synthesis. *trans*-Aconityl acid **6** is more reactive than the corresponding cis isomer and may be incorporated into the polymer chain.

Once within the polymer mainchain, the cis double bond has a lower propensity to isomerise to the trans configuration. Methylation of aconityl anhydride at position C-2 to give **12** has a threefold effect that decreases the chance of side reactions with water, stabilizes







the cis form and decreases the reactivity of the trans isomer. Together these effects may ensure production of polymer displaying an enhanced degradation profile derived from the cis double bond. These studies have aided the understanding of the aconityl element within polymers we are developing. Further investigations (modelling, synthesis and toxicity studies) to optimise the properties of these biomedical polymers are currently ongoing.

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