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# Structure and catalytic activity of some soluble polyethylene glycol–peptide conjugates

David R. Kelly,[a,*] Tam T. T. Bui,[b] Eva Caroff,[c] Alex F. Drake[b] and Stanley M. Roberts[c]

[a]*Department of Chemistry, Cardiff University, PO Box 912, Cardiff, Wales CF10 3TB, UK*
[b]*Department of Pharmacy, Franklin Wilkins Building, Kings' College, London SE1 9NN, UK*
[c]*Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, UK*

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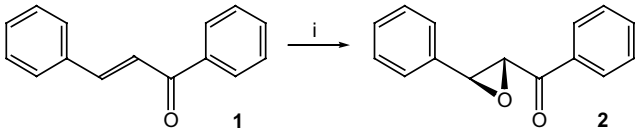
**Abstract**—Soluble catalysts for the Juliá–Colonna asymmetric epoxidation reaction have been constructed in three different ways, using $NH_2$-PEG-OMe as the support system: suitable solvents have been identified and it is shown that the degree of helicity of the conjugates correlates with the extent of conversion and (to a lesser extent) the enantioselectivity of epoxidation.


The asymmetric epoxidation of α,β-unsaturated ketones such as chalcone **1** into the corresponding epoxide **2** using synthetic peptides (such as poly-(L)-leucine, PLL) has become a well-established method.[1] The original procedure, introduced by Juliá and co-workers[2] and popularised by Lantos and co-workers,[3] Bezuidenhoudt and co-workers[4] and others, used insoluble peptides as heterogeneous catalysts in a triphasic system (aq $H_2O_2$, substrate/toluene, peptide). The use of urea hydrogen peroxide complex (UHP) in THF removed the need for water and yielded a biphasic system. Recently the Liverpool group,[5] and a team from Degussa,[6] have prepared THF-soluble asymmetric epoxidation catalysts by appending peptide chains to diaminopolyethylene glycol ($H_2N$-PEG-$NH_2$) to give bilobal conjugates **3**.




**Scheme 1.** Reagents and conditions: (i) catalyst, UHP (1.1 equiv), DBU (1.1 equiv), 3 h, rt.

The discovery of these homogeneous catalysts is immediately useful in two ways. First, they are the catalysts of choice for employment in a continuous flow reactor.[6] Secondly it is proving to be much easier to investigate the physical properties of the soluble catalysts, in contrast to the corresponding insoluble, gel-like 'free' peptides.

In this study we have used monoamino-monomethoxy-polyethylene glycol ($H_2N$-PEG-OMe) to prepare monomeric THF soluble peptides, with the intention that these species should be amenable to analysis by enzymatic assay techniques in a monophasic system. Thus, MeO-PEG-$NH_2$ (average MW ca. 5000) was stirred with 15 equiv of (L)-leucine-*N*-carboxyanhydride [(L)-Leu-NCA] to give the peptide conjugate **4**.[7]

| | |
|---|---|
| H-[(L)-Leu]$_n$-NH-PEG-NH-[(L)-Leu]$_n$-H | **3** |
| H-[(L)-Leu]$_{15}$-NH-PEG-OMe | **4** |
| H-[(L)-Ala]$_{15}$-NH-PEG-OMe | **5** |
| H-[(L)-Ala]$_8$-[(L)-Leu]$_8$-NH-PEG-OMe | **6** |
| H-[Aib]$_8$-[(L)-Leu]$_8$-NH-PEG-OMe | **7** |
| H-[(L)-Leu]$_8$-[(L)-Ala]$_8$-NH-PEG-OMe | **8** |

Physical measurements on the new catalysts were envisaged to involve different concentrations of reagents and/or catalysts in a variety of solvents. Thus it was important to assess the effect of these parameters on catalyst activity. First it was shown that the amount of solvent used in the standard protocol had no effect on the stereoselectivity of the reaction. Varying the chalcone concentration from 6 to 120 mM invariably gave epoxide with 97% ee (see Table 1, entry 1).[8]

* Corresponding author. Tel.: +44-(0)2920-874063; fax: +44-(0)2920-874030; e-mail: kellydr@cardiff.ac.uk

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**Table 1.** Effect of solvent on homogeneous Juliá–Colonna oxidation of chalcone **1** (80 mM) to chalcone epoxide **2**, catalysed by H-[(L)-Leu]$_{15}$-NH-PEG-OMe **4**

| Entry | Solvent | Conversion (%) | Ee (%) |
|---|---|---|---|
| 1 | THF | 95 | 97 |
| 2 | DME | 99 | 96 |
| 3 | Toluene | 51 | 94 |
| 4 | $CH_3CN$ | 97 | 45 |
| 5 | $CHCl_3$ | 78 | 41 |
| 6 | THF–$CH_3CN$, 2.8:1 | 94 | 88 |

Conditions as Scheme 1.

Changing the solvent to dimethoxyethane (DME) gave an excellent conversion to chalcone epoxide **2** possessing high ee (Table 1, entry 2). Using toluene as solvent resulted in a slower reaction, but the product still showed a high ee (entry 3). In contrast acetonitrile (entry 4) and chloroform (entry 5) supported fast reactions but the product was formed in only 45% and 41% ee, respectively. Mixing THF and acetonitrile in the ratio 2.8:1 resulted in a fast reaction (87% conversion in 70 min) to generate chalcone epoxide **2** with 88% ee (entry 6). Still faster conversions (77% conversion in 15 min) could be attained using excess peroxide (1.75 equiv) and DBU (1.59 equiv) under the latter conditions, with no adverse effect on the enantiomeric excess.

The conformations of the polyleucine chains in the polymer **3** have been investigated by solution phase IR spectroscopy. It was found that the α-helical content of the leucine chains increased on increase in chain length.[5] A similar relationship was mooted by Juliá and Colonna with respect to the insoluble catalyst,[9] while Ohkata et al. have shown that Boc-[(L)-Leu]$_6$-Aib-[(L)-Leu]$_6$OBn[10] possesses 'a high degree of helical conformational structure'.[11]

In order to study the relationship of helicity and catalytic activity more rigorously, a series of new polymers were prepared. Thus, MeO-PEG-$NH_2$ (average MW ca. 5000) was stirred with 15 equiv of (L)-alanine-NCA, to give the polymer **5**. Similarly MeO-PEG-$NH_2$ was stirred with 8 equiv of (L)-leucine-NCA for 24 h: to this mixture was added 8 equiv of (L)-alanine-NCA or 8 equiv of Aib to produce block polymers **6** or **7**.[12] The block polymer **8** was obtained in a similar fashion.

Each of the polymers was tested for its ability to catalyse the oxidation of chalcone **1** to (2*R*,3*S*)-epoxychalcone **2** (Scheme 1). The secondary structure of the polymers was determined by circular dichroism in acetonitrile solution using an APL PiStar-180 CDF spectrometer (Fig. 1 and Table 2).

From the data in Table 2, several features are of interest. Firstly, the polyleucine conjugate **4** is largely the α-helical conformer and is an excellent epoxidation catalyst, whereas the polyalanine conjugate **5** has the lowest α-helical content and is the poorest catalyst amongst the five polymers.[14]

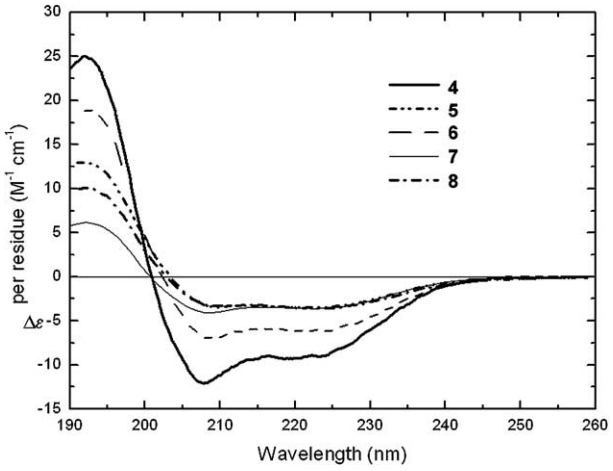



**Figure 1.** Far-UV CD spectra of MeO-Peg-NH-amino acids **4**–**8** (5 mg $mL^{-1}$) in $CH_3CN$. All spectra have been normalised for concentration per residue and path length. Secondary structure analysis was undertaken using standard chemometric routines.[13]

The sequence complementary isomers **6**, **8** provide an interesting contrast; both give chalcone epoxide **2** with good to excellent enantiomeric excesses, but the $Ala_8$-$Leu_8$ isomer **6** is much more catalytically active than the $Leu_8$-$Ala_8$ isomer **8** and has a much higher proportion of the α-helical conformer. In the same way, the $Aib_8$-$Leu_8$ conjugate **7** has a similar helicity to the $Leu_8$-$Ala_8$ isomer **8**, which results in a similar degree of conversion and enantiomeric excess. This result is all the more remarkable, because half of the peptide consists of configurationally achiral Aib units and hence a proportion of the α-helical conformer presumably results from conformationally induced macromolecular chiral amplification.[16] This effect may be further augmented by the preference for PEG to adopt a helical conformation.[17] The helical conformation of the PEG chain is not detectable by CD, but it may affect the preference for helicity of all the peptide conjugates. The measured degree of α-helicity of the peptide conjugates **4**–**6**, **8** is much higher than that predicted by the AGADIR programme[15] (Table 2, penultimate column; standard deviation of prediction 6%). Nevertheless the rank order, the large difference in α-helicity between the $Leu_{15}$- and $Ala_{15}$-conjugates **4**, **5**, and the trend for the $Ala_8$-$Leu_8$ conjugate **6** and the $Leu_8$-$Ala_8$ isomer **8** are correctly predicted. A *C*-amidated decapeptide only has seven intra-residue hydrogen bonds, therefore a small change in hydrogen bonding, can have an enormous effect on α-helicity, this can be due to a single extra hydrogen bond or a small increase in the strength of the bonds due to ionic or temperature effects. To mitigate against this extreme sensitivity, α-helicity was also calculated using AGADIR for the homologous series of peptides corresponding to the catalysts **4**–**6**, **8**. The closest fit to the experimental data and the homologue giving this fit is shown in the last column of Table 2. By this measure the predictions are remarkably accurate. The $Leu_{15}$-catalyst **4**, which has an average composition of 10 residues is predicted to contain 11 residues and both the $Ala_8$-$Leu_8$ conjugate **6** and the $Leu_8$-$Ala_8$

**Table 2.** Catalytic activity and secondary structure of peptide-NH-PEG-OMe catalysts **4**–**8**, ranked by measured % α-helicity

| | Formation of chalcone epoxide **2** (%) | | Secondary structure (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Measured | | | Predicted α-helicity[15] | |
| Polymer | Conv.[a] | Ee | α-Helix | β-Sheet | Other | 10-mers[b] | $X_n(Y_n)$-mers[c] |
| **4** | >95 | 97 | 86 | 14 | 0 | 72 | 87, $Leu_{11}$ |
| **6** | 58 | 97 | 63 | 24 | 13 | 8 | 67, $Ala_7Leu_7$ |
| **7** | 20 | 78 | 39 | 14 | 45 | — | — |
| **8** | 16 | 88 | 36 | 25 | 39 | 4 | 32, $Leu_7Ala_7$ |
| **5** | 10 | 28 | 32 | 39 | 29 | 5 | 35, $Ala_{16}$ |

Conditions as Scheme 1.

[a] Conversion.

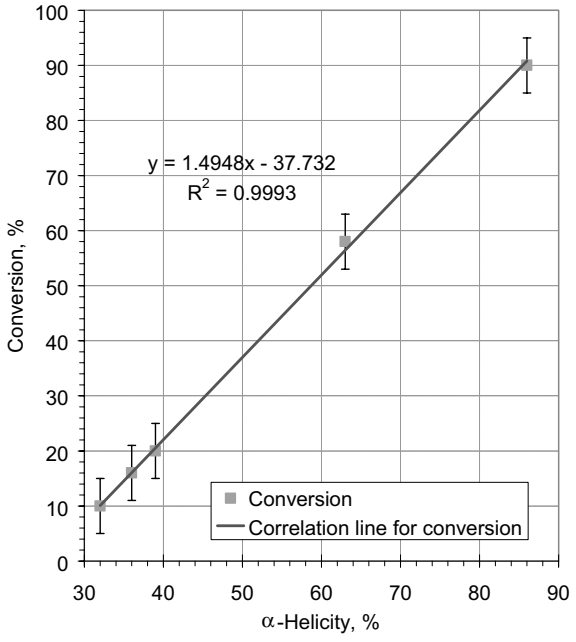
[b] Predicted α-helicity for peptides containing 10 residues.[7]

[c] 'Best fit predictions' for α-helicity. The sequences indicate the homologues ($n = 10$–16 **4**, **5** and $n = 5, 5$–$8, 8$ **6**, **8**), which most closely fit the measured α-helicity.

isomer **8** are predicted to contain 2 × seven residues. The worst case is $Ala_{15}$-catalyst **5**, which is predicted to contain 16 residues. Overall the catalysts show higher helicity than expected, which may be a consequence of a templating role by the PEG moiety.

The seeming ability of PEG to promote α-helicity, may be of benefit for self-organisation of other catalysts. In the data set as a whole, conversion can be predicted almost perfectly from α-helicity (Graph 1), whereas enantiomeric excess increases roughly with increasing α-helicity, but the span of ee values does not allow meaningful conclusions to be drawn.

The preparation of conjugates **3**–**8** by statistical polymerisation is experimentally very simple, however, they are formed as polydisperse mixtures with a Gaussian distribution of components. This causes no significant problem for medium or long chain peptides, but does not allow meaningful kinetic data to be extracted from short peptides, when the shorter congeners are expected to have low or no catalytic activity. Consequently the homogeneous conjugates H-[(L)-Leu]$_n$-NH-PEG-OMe ($n = 4$–7) **9**–**11**, **13**, were prepared by stepwise DCC mediated coupling of Fmoc-(L)-leucine.[18] The hexaleucine conjugate **12** was also prepared in a single step by coupling Fmoc-[(L)-Leu]$_6$-OH in the same way. This is the first time a soluble Juliá–Colonna epoxidation catalyst has been formed from a preformed peptide. For comparison, H-[(L)-Leu]$_5$ NH-PEG-OMe **14** was also prepared by the statistical polymerisation method using 8 equiv of (L)-leucine-NCA.[7] The conjugates **9**–**14** were compared in their effectiveness as catalysts for the epoxidation of chalcone **1** (Table 3).




**Graph 1.** Conversion as a function of α-helicity ranked by %ee for polymers **4**–**8**. Error bars, conversion ± 5%.

It is clear from these results that chalcone epoxide **2** showing a good enantiomeric excess is formed using soluble catalysts having chains as short as five leucine residues and that the rate of epoxidation and conversion increases monotonically as the length of the polypeptide chain increases.[19] The difference in results between the pentaleucine conjugate **10** and the statistical mixture **14**, equate to a 3-fold increase in formation of the major enantiomer, and a 4.4-fold increase in formation of the minor enantiomer, which reflects the higher rate and enantiomeric excess achievable with slightly longer conjugates. The differences in conversion and enantiomeric excess between the hexaleucine conjugates, prepared by stepwise **11** and single coupling **12** are at the limits of the reproducibility of the analytical techniques (conversion ±5% and ee ±3%). Finally the stereochemical fidelity of the heptaleucine conjugate **13** is indistinguishable from that of the leucine 15-mer conjugate **4**, albeit that the conversion is much lower.

**Table 3.** Comparison of H-[(L)-Leu]$_n$-NH-PEG-OMe, $n = 4$–7 catalysts in the epoxidation of chalcone **1** to chalcone epoxide **2**

| Polymer | *n* | Conversion (%) | Ee (%) |
|---|---|---|---|
| **9** | 4 | 6 | 42 |
| **10** | 5 | 10 | 87 |
| **11** | 6 | 19 | 90 |
| **12** | 6, Single coupling | 28 | 95 |
| **13** | 7 | 31 | 96 |
| **14** | 5, Average | 30 | 81 |

Conditions as Scheme 1.

These data parallel those for the triphasic and biphasic catalytic systems and provides some assurance that kinetic studies[8] of the soluble peptide conjugate catalyst will be *mechanistically* relevant to the other systems.

In summary, soluble epoxidation catalysts can be prepared by appending short peptides to PEG. The rate/ee of epoxidation correlates with the helicity of the polypeptide and increases with increasing chain length.

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