Monodisperse liquid crystalline peptides

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Solid phase peptide synthesis (SPPS) has been used as a synthetic tool to prepare monodisperse mesogen–oligopeptide conjugates with novel molecular architectures. Building upon previous success in this area, we have extended our methodologies to include a mesogenically substituted L-glutamic acid residue, and incorporated this derivative, as well as the L-lysine derivative previously reported, into a variety of new structures. Some novel liquid crystalline materials have been discovered. Both α-helical and β-sheet secondary structures have been explored as alternative scaffolds for the pendant mesogenic groups. The helical systems show most promise in terms of their ease of synthesis and handling, but at the relatively lower levels of mesogenic substitution studied so far, compared to the previously reported homo-oligopeptide materials, mesophases are not observed. The β-sheet materials prepared are rather insoluble and too high melting to have any practical value as thermotropic materials without further manipulation of their structures.

For a number of years, one of the major goals in polymer science has been to prepare high polymers with narrow polydispersities and pre-determined molecular mass. This synthetic control would allow tailoring of physical properties and lead to a widening of polymers' potential usefulness, and has now been achieved successfully for a number of polymer classes, using a variety of different methodologies. This synthetic aim is no different in the field of liquid crystalline polymers (LCPs), where it has been shown that the degree of polymerisation can exert a significant influence upon the mesophase stability, as well as the nature of the mesophase itself.[1] It is also known that polydisperse LCPs can display multi-component mesophases.[2] As a result of these effects, many of the methods for controlled polymerisation which are now well known in the literature have been applied to the synthesis of LCPs, with some success. These include cationic,[1,3] anionic,[4,5] group-transfer[6,7] and ring-opening metathesis[8,9] polymerisation studies.

As outlined in a preliminary communication,[10] we became interested in applying the synthetic control offered by solid phase methods in the preparation of side-chain liquid crystalline oligopeptides and polypeptides. This approach is very attractive in terms of preparing truly monodisperse materials, but also, by the very nature of the technique, it offers unprecedented control over the primary and secondary structures of the peptide backbones, promising entry into structurally interesting and unusual materials. Although to date solid phase methods have been used extensively for the routine synthesis of peptides, and have become central to the preparation of libraries of compounds by combinational methods,[11–13] it had never before been reported, to our knowledge, for the premeditated synthesis of structurally well-defined liquid crystalline materials prior to our preliminary communication.[10]

In the latter, we described the successful evolution of a solid phase approach to the synthesis of L-lysine oligomers substituted in the side-chain with a mesogenic group. We detailed the preparation of the dimer, the trimer and the tetramer of a mesogenically substituted L-lysine derivative, and showed that these did indeed display thermotropic behaviour, as anticipated. In this full paper we give further details, and will show how we have extended our methodologies to include other primary sequences, utilising both the L-lysine derivative reported previously, as well as a mesogenically substituted amino acid based on L-glutamic acid. Amino acid sequences which support either α-helical or β-sheet secondary structures are also explored as alternative scaffolds for the mesogenic groups.

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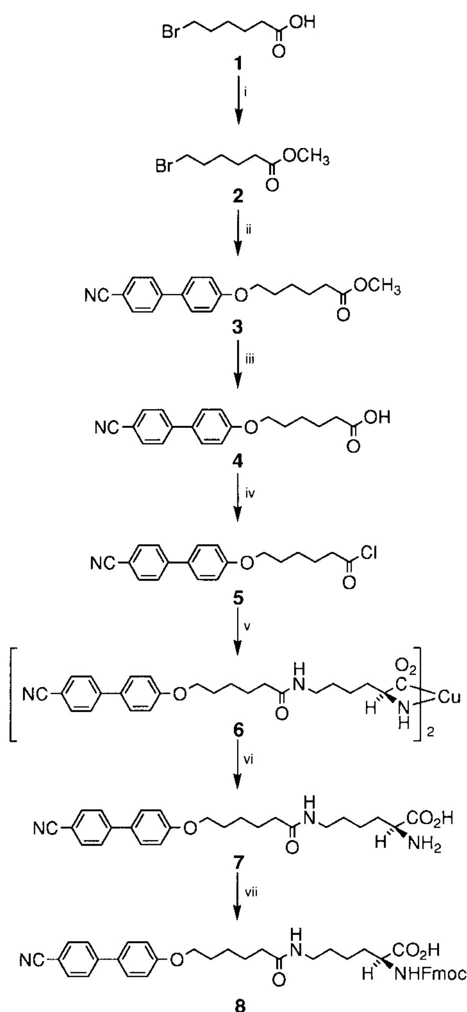
Results and Discussion

Synthetic concepts and approaches

At the beginning of this current study, we identified two possible routes into structurally well-defined, side-chain liquid crystalline peptides, which were directly analogous in concept to those utilised in the preparation of side-chain functionalised polymers. Thus one could modify, by attachment of a mesogen, a pre-formed peptide which has been prepared by solid phase means. Alternatively, an amino acid derivative appropriately functionalised with a mesogen could be utilised in the solid phase synthesis. Although the former is certainly attractive in several respects, it was the latter approach which we adopted because it offered potentially the greatest degree of synthetic control.

In the design and synthesis of functionalised amino acids suitable for our purposes, there were a number of aspects which were considered, to give our systems synthetic and structural versatility, whilst maximising the likelihood of mesophase formation. These included not only their ease of synthesis and their stability, but also their potential to facilitate the formation of ordered structure over and above that of the liquid crystal phase. We concentrated upon the α-amino acids because of their ready commercial availability in an optically pure form, their well established chemistry and their interesting secondary structure forming abilities.[14] Bearing in mind the implications of the 'spacer concept'[15] for side-chain liquid crystalline polymers, as well as the structures of certain polydisperse liquid crystalline polypeptides already reported in the literature, we felt that the incorporation of a flexible spacing unit in all our designs was necessary to promote liquid crystallinity. Mesogenic groups based on 4-cyano-4′-hydroxybiphenyl were employed throughout, because these have been extensively studied in both low molecular mass materials and in polymers, and are reasonably stable under a variety of conditions.



Scheme 1 *Reagents and conditions*: i, MeOH, H_2SO_4; ii, K_2CO_3, $NCC_6H_4C_6H_4OH$; iii, KOH; iv, $SOCl_2$; v, $CuLys_2$, NaOH; vi, EDTA·2Na; vii, Fmoc-Cl, Na_2CO_3

Synthesis of amino acid derivatives

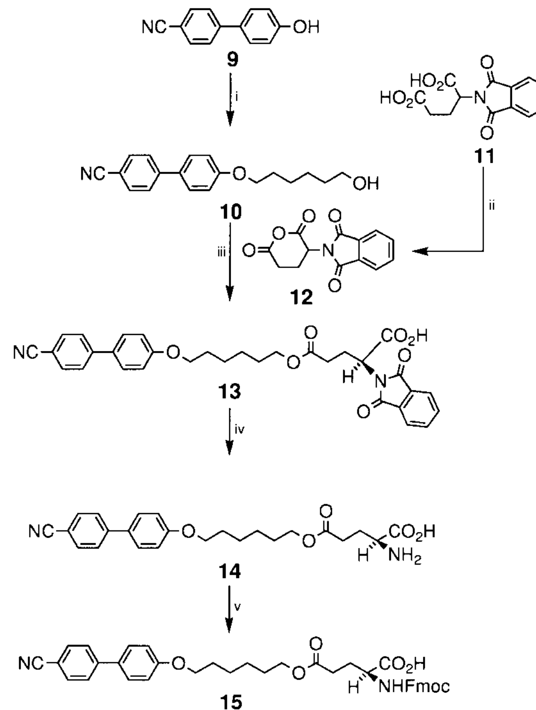
The L-lysine derivative **8** was prepared by the route shown in Scheme 1. 6-(4-Cyanobiphenyl-4′-yloxy)hexanoic acid **4** was prepared from 6-bromohexanoic acid **1** according to a literature procedure.[16] In the key step of the synthesis, the acid chloride of **4** was coupled selectively to the ε-amino group in the L-lysine copper complex using interfacial reaction conditions, in a fashion similar to that previously reported by Guéniffey for the reaction of long-chain acid chlorides with the L-lysine copper complex.[17] Thereafter, decomplexation followed by fluorenylmethoxycarbonyl (Fmoc) protection gave **8** in an overall yield of 14%, in eight steps.

The L-glutamic acid derivative **15** was prepared *via* the route shown in Scheme 2. 6-(4-Cyanobiphenyl-4′-yloxy)hexanol **10**[18] and *N*-phthaloyl-L-glutamic anhydride **12**[19] were prepared according to literature procedures, and coupled under conditions similar to those employed by Feijen[20] for a related system, to give **13**. Phthaloyl deprotection[21] followed by Fmoc protection gave **15** in an overall yield of 10% in five steps.

Solid phase synthesis of oligopeptides

Homo-oligopeptides. As detailed elsewhere,[10] the dimer, trimer and the tetramer H-[Lys(M)]$_n$-NH_2, $n=2$ (**16**), 3 (**17**) and 4 (**18**)‡ of the L-lysine derivative were successfully prepared.

‡Throughout, M = 6-(4-cyanobiphenyl-4′-yloxy)hexanoyl for lysine derivatives and 6-(4-Cyanobiphenyl-4′-yloxy)hexyl for glutamic acid derivatives.



Scheme 2 *Reagents and conditions*: i, $Cl(CH_2)_6OH$,KOH; ii, Ac_2O; iii, toluene, heat; iv, $PhNHNH_2$, Bu_3N; v, Fmoc-OSu, Na_2CO_3

The efficiency with which the modified amino acid coupled to the growing peptide chain was particularly notable, and yielded materials which did not require further purification. This high purity was borne out by the sharp thermal transitions which were observed during heating and cooling cycles. Hot-stage polarised optical microscopy revealed thermotropic phases for all three materials which are visually of indistinct texture, but which we believe to be the nematic phase, since the samples displayed shear birefringence as well as showing no fan texture typical of some smectic phases.

The peptides **16–18** are considered as 'uncapped' since the N-terminus is present as the free amine. We realised that perhaps an opportunity existed for modifying the mesophases and/or the transition temperatures through careful control of the capping group at the N-terminus, and so proceeded to prepare the *N*-acetyl derivatives Ac-[Lys(M)]$_n$-NH_2, $n=2$ (**19**), 3 (**20**) and 4 (**21**) of the three peptides. To our surprise, acetylating the N-terminus completely destroyed the mesogenic properties of the peptides. We believe that this may be due to a hydrogen bonding interaction between the amide group at the N-terminus and the amide group in the side-chain, but we are currently carrying out further capping studies to probe this interesting effect further.

In a similar fashion to the L-lysine system we have also begun to construct the L-glutamic acid series of homo-oligopeptides. In the first instance we have prepared the dimer, with the N-terminus either acetylated or present as the free amine X-[Glu(M)$_2$-NH_2, X = H (**22**) or Ac (**23**). Again, the 'as cleaved' materials were of apparently high purity, and this time both the acetylated and the uncapped peptide displayed a mesophase, which was readily identifiable as the nematic phase in both cases. We were unable to crystallise **22**, and indeed this oily compound was observed to be birefringent at room temperature. Further extension of this series to include the higher analogues will be required to establish structure–property relationships, and also to ascertain the effect of developing secondary structure upon the liquid crystalline behaviour as the main-chains become longer.

The fact that the acetylated derivative **23** was liquid crystalline in this case can be considered as lending some supporting

evidence to the reasoning behind why the acetylated lysine derivatives **19–21** were not liquid crystalline, since **23** has an ester functionality in the side-chain, and not an amide.

Targetted α-helical peptides. In developing the concept of monodisperse liquid crystalline peptides with additional secondary structure imposed by the peptide backbone, we have become interested in the preparation of peptides where the main-chain is in the α-helical conformation, since this structural feature is common to many natural peptides and proteins, and is present in virtually all polydisperse polypeptides which display mesomorphic behaviour.[22] To succeed in this goal, one must have an understanding of the structural parameters which dictate secondary structure formation. These include the primary sequence of the peptide,[23] the length of the peptide chain,[24,25] the presence or otherwise of substituents at the N- and C-termini[26,27] and other factors such as the ability to form salt bridges or disulfide bridges which can stabilise secondary structures.

Our first α-helical peptide target was the dodecapeptide **24**, Ac-AlaAlaLys(M)AibAlaLys(M)AlaAibAlaLys(M)AlaAla-NH_2, which contains seven alanine (Ala) residues, two α-aminoisobutyric acid residues (Aib) and three mesogenically substituted L-lysine residues [Lys(M)]. We considered the level of mesogenic substitution to be rather lower (25% of amino acid residues are mesogenic) than would be considered ideal for the formation of liquid crystalline phases, but nonetheless **24** provided an ideal initial target to assess the feasibility of the approach.

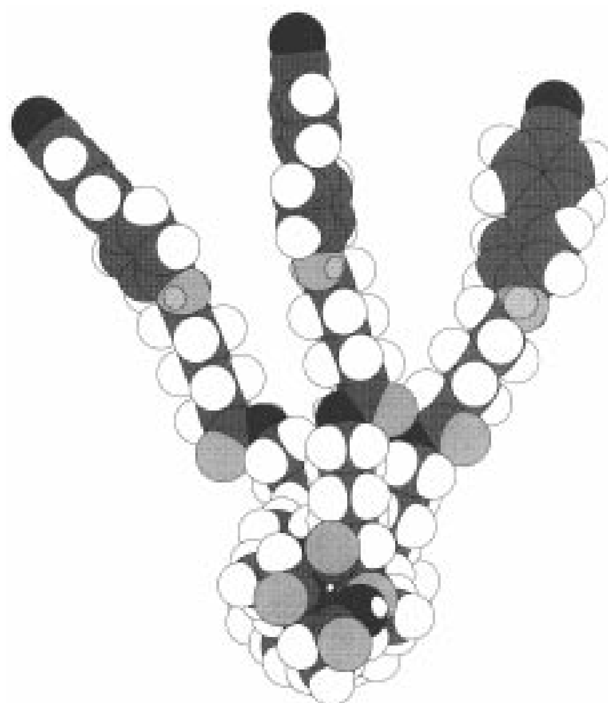


Fig. 1 A molecular model of the dodecapeptide **24**, showing the three mesogenic groups pendant on one side of the α-helix. The main axis of the α-helix is normal to the plane of the page.

The amino acids in the sequence were chosen on the basis of their α-helix forming propensity; alanine is well known for its helix forming abilities,[23] as is the non-natural amino acid α-aminoisobutyric acid.[26,28] The conformational parameters of the non-natural Lys(M) residue were not known, but it was anticipated that it would either be a weak helix former or relatively neutral conformationally, and hence quite readily sit in a helical conformation in the presence of other strong helix formers. To our advantage, not only do Aib residues show strong helical conformation preferences, but they also inhibit the formation of β-sheets. The C-terminus and the N-terminus of **24** were capped as the acid amide and the acetyl derivative respectively, to enhance the solubility of the peptide in organic solvents and also to enhance the likelihood of helix formation.[27,29] Crucially, the positions of the Lys(M) residues within the sequence were carefully chosen so that all three of the mesogenic groups would lie on approximately the same side of the α-helix (Fig. 1). We believed that this arrangement of side-chains offered the best prospect for mesophase formation at this level of mesogenic substitution.

After purification by reverse phase HPLC and precipitation from methanol, which in our hands has often proved to be helicogenic,[22] **24** was indeed shown to adopt an α-helical conformation, as confirmed by the absorption bands present at 1659 (amide I) and 1541 cm^{-1} (amide II) in the solid state FTIR spectrum.[30] Upon heating, it melted directly into the isotropic liquid state at 196 °C, and crystallised directly from the melt upon cooling. That is, no thermotropic behaviour was observed, as perhaps anticipated. Nonetheless we at least demonstrated that this was a feasible synthetic approach towards these well-defined helical structures.

As a second α-helical peptide target, we selected the tetradecapeptide **25**, Ac-AlaAlaGlu(M)AibGlu(M)AlaGlu(M)Glu-(M)AiBGlu(M)AlaGlu(M)AlaAla-NH_2, which again contains predominately Ala and Aib residues. The level of mesogenic substitution is significantly higher (*ca.* 43% of residues are mesogenic) than for **24** to favour mesophase formation, and because of this we elected to utilise the L-glutamic acid derivative because the ability of L-glutamic esters themselves to support an α-helix is well known.[22] The mesogenic residues within the primary sequence were arranged such that three would lie on one side of the helix, with the remaining three on the opposite side. This, we felt, was the most favourable arrangement of side-chains at this level of mesogenic substitution, in terms of potential mesophase formation. This arrangement is most easily visualised in the molecular model (Fig. 2).

After purification by reverse phase HPLC and precipitation from the helicogenic solvent acetonitrile,[22] the conformation of **25** was investigated using infrared spectroscopy. Bands were observed at 1668 and 1543 cm^{-1}.[30] The former is outside the range for the amide I band of a helix and instead suggests **25** is folded into a series of beta turns or loops. Thermal studies indicated that the material did not exhibit any thermotropic phases, with melting from the crystalline to the isotropic liquid being observed at 75 °C, and crystallisation directly from the isotropic melt occurring upon cooling. This could be because the level of mesogenic substitution is simply not high enough to sustain a mesophase or because the poorly defined peptide conformation is unfavourable for ordering. Accordingly, we are currently working towards more robust helical systems where the mesogenic content is still higher.

Targetted β-sheet peptides. To date, all of the polydisperse liquid crystalline polypeptides reported in the literature have been based upon α-helical main-chains. We were very interested, therefore, in exploiting the opportunities lent to us by our methodologies to prepare materials with different secondary structures. Accordingly, a series of six peptides (**26–31**) based upon a simple repeat unit X-[ValLys(M)]$_n$-NH_2; X=H or Ac; n=1, 2 or 3 was prepared, designed to adopt an extended β-sheet conformation. Valine (Val) has strong β-sheet preferences. Every second residue in the sequence was mesogenically substituted.

The solid phase synthesis of these peptides proceeded reasonably well, but the presence of deletion peptides was apparent in the mass spectra of the crude samples of the higher ($n>2$) analogues. The low solubility of these materials in a range of solvents meant that satisfactory purification was not possible,

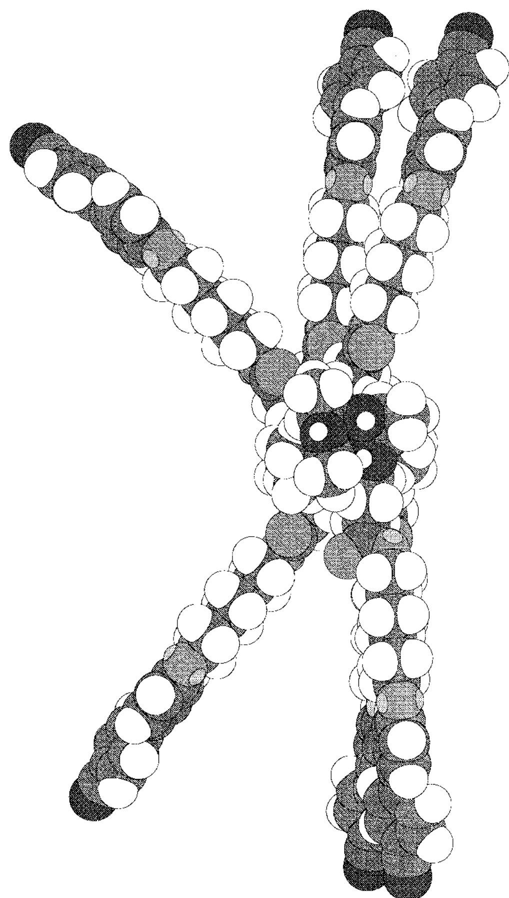


Fig. 2 A molecular model of the tetradecapeptide **25**, showing three mesogenic groups pendant on one side of the α-helix, and the remaining three pendant on the opposing side. The main axis of the α-helix is normal to the plane of the page.

and as a result the thermal measurements were carried out on the 'as cleaved' materials.

FTIR spectroscopy showed that an extended chain (β-sheet) conformation had indeed been adopted in every case, as shown by the amide I and amide II absorption bands present at *ca.* 1635 and *ca.* 1545 cm^{-1} respectively in each sample.[30] However, thermal studies revealed that all these materials either melted or decomposed at high temperatures (typically > 300 °C) without entering into a liquid crystal phase. This was deemed too high to be of any practical value, so we are currently pursuing structural modifications aimed at lowering the melt temperatures and allowing fluid phases to be accessed at more reasonable temperatures. In principle, these modifications, which involve disrupting the hydrogen bonding, should also make these materials more soluble and hence easier to purify and handle.

Summary and Prospects

In conclusion, we have demonstrated that the solid phase approach to the synthesis of monodisperse liquid crystalline oligopeptides is indeed very powerful, and has allowed us to prepare a number of interesting mesogen–peptide conjugates of unique structure; the materials prepared to date are summarised in Table 1. In the first instance we prepared the dimer, the trimer and the tetramer of a mesogenically substituted L-lysine derivative, and found all of these to be liquid crystalline when the N-terminus was uncapped. We are currently extending this series to include the higher analogues, whilst at the same time developing an alternative series based upon an L-glutamic acid derivative. We have also shown that we can readily prepare much longer peptides, containing a number of mesogenic units, by this approach (up to 14 residues in length), and control their secondary structures through appropriate design of the amino acid sequence. Although we have not yet been able to prepare longer peptides which are thermotropic, either because the level of mesogenic substitution was too low or the materials were too high melting, we are nonetheless currently pursuing a number of approaches which will undoubtedly make this possible.

Table 1 Summary of the peptides prepared, showing their composition and their thermal behaviour

peptide	no. of amino acids in chain	mesogenic content (%)	thermal behaviour
	homo-oligopeptides-L-lysine series		
16[a]	2	100	K 142 °C LC 173 °C I
17[a]	3	100	K 163 °C LC 190 °C I
18[a]	4	100	K 204 °C I 175 °C LC
19[b]	2	100	mp 116 °C
20[b]	3	100	mp 120 °C
21[b]	4	100	mp 118 °C
	homo-oligopeptides-L-glutamic acid series		
22[a]	2	100	N 71.6 °C I
23[b]	2	100	K 109 °C I 105 °C N 60 °C K
	α-helical peptides		
24	12	25	mp 196 °C
25	14	~43	mp 75 °C
	β-sheet peptides		
26–31	4, 6 or 8	50	mp ~300–350 °C (decomp.)

[a]Uncapped, [b]Capped.

Experimental

Materials

All materials used in this study were commercial samples, and were used as supplied unless otherwise stated. For all the solid phase syntheses, the amino acids and coupling reagents employed were of peptide synthesis grade, and were supplied by Novabiochem. *N*,*N*,-Dimethylformamide (DMF) (Rathburn, peptide synthesis grade), piperidine (Rathburn, peptide synthesis grade), *N*,*N*-diisopropylethylamine (Aldrich, 99%) and trifluoroacetic acid (TFA) (Rathburn, gas phase sequencer grade) were also used as supplied. The resin support used throughout (PR500, 0.36 mmol g^{-1} nominal loading) was supplied by Novabiochem. All chromatographic solvents were distilled prior to use, and other solvents purified, where appropriate, using standard procedures.

Instrumentation

^{1}HNMR spectra were recorded on a 250 MHz Bruker WM-250 spectrometer. FTIR spectra were recorded either on a UNICAM Matteson 1000 spectrometer or on a Nicolet Impact 400D spectrometer. Elemental microanalysis was carried out by the Microanalytical Laboratory at the University of Strathclyde. Melting points were recorded on a Gallenkamp melting point apparatus, and are uncorrected. HPLC was performed on a C_8 column (25 × 4.6 mm, packed with 5 μm Spherisorb ODS2, supplied by Anachem) using Gilson 306 pumps and a Gilson 117 UV detector. ES–MS spectra were recorded on a Fisons VG Platform spectrometer. DSC was carried out on a Perkin-Elmer DSC 7 differential scanning calorimeter. Molecular modelling was carried out using MACROMODEL, Version 4.5, on a Silicon Graphics Indigo work-station.

Solid phase synthesis of peptides

Oligopeptides were synthesised on a Novasyn Crystal solid phase peptide synthesiser using Novasyn PR500 resin and PyBOP coupling chemistry. Typically, each residue was double coupled using a two-fold excess of amino acid. Cleavage of the peptide from the resin was with 10% TFA in CH_2Cl_2 for 90 min.

Liquid crystal characterisation of peptides

Hot stage polarised optical microscopy was performed either on an Olympus CH-2 microscope fitted with a Mettler FP-5 hot-stage, JVC TK-1085E colour video attachment and a Sony UP-3000P colour video printer, or on an Olympus Vanox microscope fitted with a Linkam TH600 hot-stage and a Linkam PR600 thermal controller. Microscope slides were pretreated with a homogeneous aligning agent.

Syntheses

L-Lysine derivative 8. *Methyl 6-bromohexanoate* **2**. 6-Bromohexanoic acid (22.00 g, 113 mmol), concentrated sulfuric acid (3 cm^3) and methanol (50 cm^3) were refluxed for 3 h and, after cooling, excess methanol was removed under reduced pressure. Portions of deionised water (100 cm^3) and chloroform (100 cm^3) were added to the colourless liquid residue, and the organic layer separated. The aqueous layer was further extracted with portions of chloroform (3×50 cm^3). The organic layers were combined, washed with 5% aqueous sodium hydrogen carbonate (100 cm^3), then water (50 cm^3), dried over magnesium sulfate and the chloroform removed under reduced pressure. The crude liquid residue was distilled under reduced pressure and **2** collected as a colourless liquid (17.16 g, 73%), bp 40 °C at 0.01 mbar. (Found: C, 40.6; H, 6.6; Br, 37.7. $C_7H_{13}BrO_2$ requires C, 40.2; H, 6.3; Br, 38.2%); ν_{max}/cm^{-1} 2947, 2865, 1740, 1436, 1362, 1255, 1202, 1173, 1122 and 1000; δ_H ($CDCl_3$) 1.40–1.52 (m, 2H), 1.57–1.76 (m, 2H), 1.78–1.98 (m, 2H), 2.33 (t, 2H), 3.38 (t, 2H) and 3.66 (s, 3H).

Methyl 6-(4-*cyanobiphenyl*-4′-*yloxy*)*hexanoate* **3**. The ester **2** (8.80 g, 42.1 mmol), 4-cyano-4′-hydroxybiphenyl (8.21 g, 42.1 mmol) and anhydrous potassium carbonate (5.82 g, 42.1 mmol) were stirred in DMF (20 cm^3) for 24 h in a flask fitted with a calcium chloride guard tube. The viscous yellow slurry which formed was poured into water (200 cm^3) and the resultant white precipitate filtered off and washed with water. The crude product was dried over phosphorus pentoxide for 48 h, and then recrystallised from 80% aqueous ethanol (175 cm^3) giving **3** as a white powder (9.90 g, 73%), mp 88–89 °C (lit.,[16] 80–81 °C). (Found: C, 74.4; H, 6.8; N, 4.3. $C_{20}H_{21}NO_3$ requires C, 74.3; H, 6.6; N, 4.3%); ν_{max}/cm^{-1} 2947, 2869, 2222, 1722, 1600, 1497, 1473, 1294, 1272, 1254, 1181, 1031 and 829; δ_H ($CDCl_3$) 1.46–1.92 (m, 6H) 2.37 (t, 2H), 3.68 (s, 3H), 4.02 (t, 2H), 6.98 (m, 2H) and 7.50–7.73 (m, 6H).

6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexanoic acid* **4**. The ester **3** 9.00 g, 27.8 mmol) and potassium hydroxide (16.00 g, 285 mmol) were stirred in ethanol (150 cm^3) for 3 h at room temp. in a flask fitted with a calcium chloride guard tube. The resultant yellow slurry was poured into iced-water (450 cm^3), the mixture allowed to warm to room temp. and then neutralised with concentrated sulfuric acid. The white precipitate was filtered off, washed with water and dried over calcium chloride. Recrystallisation from ethanol (400 cm^3) gave **4** as a white powder (5.73 g, 67%), K 163.7 °C I 157.1 °C N 134.3 °C (from DSC) (lit.,[16] mp 165 °C) (Found: C, 73.6; H, 6.2; N, 4.1. $C_{19}H_{19}NO_3$ requires C, 73.8; H, 6.2; N, 4.5%); ν_{max}/cm^{-1} 2942, 2869, 2229, 1703, 1601, 1445, 1251, 1183, 1076, and 820; δ_H ([$^2H_8$]THF) 1.30–1.88 (m, 6H), 2.25 (t, 2H), 4.04 (t, 2H) and 6.98–7.88 (m, 8H).

6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexanoyl chloride* **5**. The acid **4** (3.00 g, 9.70 mmol) was stirred with thionyl chloride (10 cm^3, 137 mmol) for 3 h at room temp. in a flask fitted with a calcium chloride guard tube, giving a clear yellow solution. This solution was then gently heated for 90 min to expel any remaining gases from solution. The excess thionyl chloride was then removed under reduced pressure, and the oily residue co-evaporated several times with dry diethyl ether. The acid chloride was then used in the next step without further purification; ν_{max}/cm^{-1} (selected band) 1805.

Copper complex of ε-[6-(4-*cyanobiphenyl*-4′-*yloxy*)*hexanamido*]-L-*lysine* **6**. To a boiling solution of L-lysine monohydrochloride (3.54 g, 19.4 mmol) in water (60 cm^3), was added basic copper(II) carbonate (9.43 g, 42.7 mmol) over a period of 10 min; the addition caused effervescence and the supernatant solution turned bright blue. The mixture was boiled for a further 10 min, cooled to room temp. and the excess copper(II) carbonate filtered off. A solution of sodium hydroxide (0.78 g, 19.4 mmol) in water (5 cm^3) was then added to the bright blue filtrate and the solution cooled to 0 °C on an ice-bath. A solution of **5** (9.7 mmol) in dry dichloromethane (DCM) (30 cm^3) was added to the rapidly stirred solution over a period of 2.5 h, the temperature being maintained at 0 °C, and the mixture then stirred for a further 4.5 h at the same temperature. The lilac coloured precipitate which formed was filtered off, washed with ethanol, water and diethyl ether, and then air-dried (4.55 g, 100% technical yield). Further purification was not carried out; ν_{max}/cm^{-1} 3430, 3306, 2936, 2865, 2225, 1620, 1604, 1541, 1496, 1390, 1291, 1253, 1181 and 822.

ε-[6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexanamido*]-L-*lysine* **7**. The copper complex **6** (94.43 g, 4.73 mmol) was stirred with 10% aqueous ethylenediaminetetraacetic acid (EDTA) disodium salt (200 cm^3) for 20 h at room temp. The precipitate which formed was filtered off and again stirred with 10% aqueous EDTA disodium salt (200 cm^3) for a further 20 h at room temp. After filtering, the solid was washed well with water and dried *in vacuo* at 50 °C for 64 h (3.70 g, 89% technical yield), mp 285 °C. Further purification was not carried out; ν_{max}/cm^{-1} 3310, 3071, 2938, 2866, 2229, 1638, 1606, 1582, 1543, 1521, 1494, 1407, 1289, 1253, 1181 and 824.

ε-[6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexanamido*]-N-*fluorenylmethoxycarbonyl*-L-*lysine* **8**. Compound **7** (3.00 g, 6.86 mmol) was suspended in a mixture of 10% aqueous sodium carbonate (60 cm^3) and dioxane (40 cm^3), and the mixture stirred for 1 h at room temp. A solution of fluorenyl chloroformate (1.95 g, 7.54 mmol) in dioxane (20 cm^3) was then added to the stirred mixture; precipitation of a white solid was observed approximately 5 min after the addition. After stirring for a further 24 h, the mixture was poured into water (75 cm^3) giving a turbid solution. Acidification to pH 3 with 20% aqueous hydrochloric acid yielded a sticky white material which was extracted into chloroform (3×150 cm^3). The organic layers were combined, washed with water (150 cm^3) and dried over magnesium sulfate. The solvent was removed under reduced pressure and the crude product recrystallised from chloroform–light petroleum, giving **8** as a white powder (2.05 g, 45%), mp 154–156 °C. (Found: C, 72.4; H, 6.4; N, 6.1. $C_{40}H_{41}N_3O_6$ requires C, 72.8; H, 6.3; N, 6.4%); ν_{max}/cm^{-1} 3327, 3065, 2942, 2862, 2224, 1751, 1694 (sh. at 1721), 1602, 1544, 1494, 1451, 1253, 1181, 821, 760 and 741; δ_H (DMSO) 1.06–1.84 (m, 12H), 2.07 (t, 2H), 3.02 (m, 2H), 3.84–4.06 (m, 3H), 4.18–4.38 (m, 3H) and 6.93–8.35 (m, 18H); M 659.8; found, *m/z* 682.5 ($M + Na^+$) and 660.7 ($M + H^+$).

L-Glutamic acid derivative 15. 6-(4-*Cyanobiphenyl-4′-yloxy*) *hexanol* **10**. 4-Cyano-4′-hydroxybiphenyl (6.43 g, 32.9 mmol), potassium hydroxide (1.85 g, 32.9 mmol) and a few

crystals of potassium iodide were dissolved in an ethanol–water mixture (4 : 1, 165 cm^3) at room temp. 6-Chlorohexanol (4.87 cm^3, 36.6 mmol) was then added, and the mixture refluxed for 24 h. After cooling, the ethanol was removed under reduced pressure, and the resultant yellow slurry filtered off on a glass sinter. The product was carefully washed with water, dilute aqueous sodium hydroxide, and then again with water. Recrystallisation from methanol (50 cm^3) gave **10** as a white powder (4.10 g, 42%), K 92.3 °C N 113.0 °C I (from DSC) (lit.,[18] K 93.5 °C N 110.9 °C I). (Found: C, 76.7; H, 6.7; N, 4.7. $C_{19}H_{21}NO_2$ requires C, 77.2; H, 7.2; N, 4.7%); ν_{max}/cm^{-1} 3289, 2943, 2868, 2226, 1602, 1494, 1473, 1291, 1252, 1183, 1072, 1012 and 829; δ_H ($CDCl_3$) 1.23–1.96 (m, 9H), 3.68 (t, 2H), 4.02, (t, 2H), 6.98 (d, 2H), 7.42–7.83 (m, 6H).

N-*Phthaloyl*-L-*glutamic anhydride* **12**. A mixture of *N*-phthaloyl-L-glutamic acid (5.07 g, 18.3 mmol) and acetic anhydride (10 cm^3, 106 mmol) was refluxed for 10 min under a dry nitrogen atmosphere. Upon cooling to room temp., a white precipitate was seen for form. Dry diethyl ether (50 cm^3) was added to the mixture, the solids filtered off, washed with a further volume of dry diethyl ether, and then air-dried. Recrystallisation from dry ethyl acetate (75 cm^3) furnished **12** as colourless crystals, with a second crop being collected after cooling the filtrate overnight in the refrigerator (3.26 g, 69%), mp 196–197 °C (decomp.) (lit.,[19] 195–196 °C). (Found: C, 60.0; H. 3.5; N, 5.4. $C_{13}H_9NO_5$ requires C, 60.2; H, 3.5; N, 5.4%); ν_{max}/cm^{-1} 1813, 1772, 1715, 1470, 1389, 1226, 1074, 1030, 974 and 723; δ_H (DMSO) 2.53–2.70 (m, 2H), 2.90–3.20 (m, 2H), 5.43–5.50 (m, 1H), 7.88–7.97 (m, 4H).

5-[6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexyl*] *hydrogen* N-*phthaloyl*-L-*glutamate* **13**. Compounds **10** (3.56 g, 12.0 mmol) and **12** (3.12 g, 12.0 mmol) were refluxed in dry toluene (300 cm^3) under a dry nitrogen atmosphere for 24 h. After cooling, the toluene was removed under reduced pressure and the oily residue purified by flash column chromatography on silica gel, using ethyl acetate as the eluent, giving **13** as a sticky yellow oil (5.34 g, 80%). (Found: C, 69.6; H, 5.4; N, 4.8. $C_{32}H_{30}N_2O_7$ requires C, 69.3; H, 5.5; N, 5.1%); ν_{max}/cm^{-1} 2939, 2863, 2225, 1776, 1720, 1603, 1494, 1468, 1391, 1251, 1178, 1114, 823 and 720; δ_H ($CDCl_3$) 1.32–1.90 (m, 8H), 2.34–2.73 (m, 4H) 3.92–4.22 (m, 4H), 4.87–5.05 (m, 1H), 6.98 (d, 2H) and 7.47–7.96 (m, 10H).

5-[6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexyl*] *hydrogen* L-*glutamate* **14**. Compound **13** (5.34 g, 9.63 mmol) was dissolved in 96% aqueous ethanol (10 cm^3). Tributylamine (2.29 cm^3, 9.63 mmol) and freshly distilled phenylhydrazine (2.84 cm^3, 28.9 mmol) were then added and the mixture refluxed for 2 h; a solid was observed to precipitate from solution approximately 15 min after reflux was attained. Methyl ethyl ketone (20 cm^3) was added, the mixture refluxed for a further 15 min, and then cooled to room temp. Glacial acetic acid (0.83 cm^3, 14.4 mmol) was added, the mixture stirred at room temp. for approximately 10 min, and the white solid filtered off. This was washed carefully with methyl ethyl ketone and then dried *in vacuo* for 7 h at 40 °C giving **14** as a white powder (1.67 g, 41% technical yield) mp 159.7 °C (from DSC). Further purification was not carried out. (Found: C, 67.1; H, 7.0; N, 6.7. $C_{24}H_{28}N_2O_5$ requires C, 67.9 H, 6.7; N, 6.6%); ν_{max}/cm^{-1} 3445, 2938, 2864, 2228, 1730, 1604, 1580, 1496, 1414, 1329, 1254, 1181 and 823.

5-[6-(4-*Cyanobiphenyl*-4′-*yloxy*)*hexyl*] *hydrogen* N-*fluorenylmethoxycarbonyl*-L-*glutamate* **15**. Compound **14** (1.63 g, 3.84 mmol) was suspended in a mixture of 10% aqueous sodium carbonate (50 cm^3) and dioxane (35 cm^3); partial dissolution was observed. To the rapidly stirred suspension was added a solution of fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (1.30 g, 3.84 mmol) in dioxane (15 cm^3), and the mixture stirred at room temp. for 23 h; precipitation from the mixture was observed 15 min after the addition. The free-flowing white coloured mass which formed was poured into water (500 cm^3), and the resulting turbid solution–suspension acidified with glacial acetic acid. The white precipitate which formed was filtered off and washed with water and diethyl ether. Purification by flash column chromatography on silica gel using ethyl acetate as the eluent gave **15** as a sticky, yellow solid (1.72 g, 69%). (Found: C, 72.9; H, 6.3; N, 4.1, $C_{39}H_{38}N_2O_7$ requires C, 72.4; H, 5.9 N, 4.3%); ν_{max}/cm^{-1} 3351, 3064, 3039, 2935, 2862, 2225, 1726, 1602, 1522, 1493, 1251, 1178, 1051, 822, 760 and 741; δ_H ($CDCl_3$) 1.32–2.70 (m, 12H), 3.73–4.58 (m, 8H), 6.82–7.83 (m, 17H); M 646.8; *m*/*z* 647.9 ($M+H^+$) and 311.2 ($M-C_{21}H_{22}NO_3+H^+$).

Resin washing protocol

Upon completion of each peptide synthesis, and prior to cleavage of the peptide from the resin, the resin–peptide conjugate was washed according to the following protocol to remove any residual traces of DMF as well as any other soluble impurities.

The resin–peptide conjugate was removed from the Novasyn Crystal reactor column, placed on a glass sinter and then washed sequentially with DMF, *tert*-pentyl alcohol, acetic acid, *tert*-pentyl alcohol, DCM and finally diethyl ether. It was then dried *in vacuo* for 8 h.

Acetylation of resin-bound peptides

Where appropriate, peptides were acetylated at the N-terminus as follows. The dry resin-peptide conjugate (20 mg) was placed in a round-bottomed flask and distilled DCM (4 cm^3) added, followed by a few crystals of dimethylaminopyridine (DMAP) and distilled acetic anhydride (1 cm^3). The mixture was gently shaken for 4 h, the resin filtered off and washed well with fresh DCM. The resin was then dried *in vacuo* for several hours.

Cleavage of peptides from the resin support

The dry resin–peptide conjugate (20 mg) was placed in a round bottomed flask and 10% TFA in distilled DCM (10 cm^3) added; the resin was observed to turn bright red shortly thereafter. The flask contents were swirled every 10 min or so, and after 90 min the resin was filtered off on a glass sinter and washed with 10% TFA in DCM (4×5 cm^3) followed by DCM (4×5 cm^3). The filtrate was concentrated under reduced pressure, and the residue then co-evaporated several times with acetonitrile (HPLC grade). Addition of diethyl ether (glass distilled grade) to the resultant oily residue yielded a mobile, white precipitate which was collected by centrifugation and then dried *in vacuo* for 8 h.

Homo-oligopeptides

Satisfactory acylation and deprotection traces were obtained for all residues:

H-[Lys(M)]$_2$-NH$_2$ 16. K 142 °C LC 173 °C I; M 856.2, *m*/*z* 857.2 ($M+H^+$).

H-[Lys(M)]$_3$-NH$_2$ 17. K 163 °C LC 190 °C I; M 1275.8, *m*/*z* 1298.2($M+Na^+$), 1276.3 ($M+H^+$), 650.0 ($M+H^++Na^+$) and 639.0 ($M+2Na^+$).

H-[Lys(M)]$_4$-NH$_2$ 18. K 204 °C I 175 °C LC; M 1695.3, *m*/*z* 870.4 ($M+2Na^+$), 867.5 ($M+H^++K^+$), 859.4 ($M+H^++Na^+$) and 848.5 ($M+2H^+$).

Ac-[Lys(M)]$_2$-NH$_2$ 19. mp 116 °C; M 898.2, *m*/*z* 921.0 ($M+Na^+$) and 899.0 ($M+H^+$).

Ac-[Lys(M)]$_3$NH$_2$ 20. mp 120 °C; M 1317.8, *m/z* 1318.6 $(M+H^+)$, 670.9 $(M+H^++Na^+)$ and 659.9 $(M+2H^+)$.

Ac-[Lys(M)]$_4$-NH$_2$ 21. mp 118 °C; M 1737.4, *m/z* 1738.6 $(M+H^+)$, 880.5 $(M+H^++Na^+)$ and 869.7 $(M+2H^+)$.

H-[Glu(M)]$_2$-NH$_2$ 22. N 71.6 °C I; M 830.1, *m/z* 830.9 $(M+H^+)$, 495.6 $(M-C_{21}H_{22}NO_3+H^+)$ and 477.7 $(M-C_{21}H_{22}NO_3-NH_2^-)$.

Ac-[Glu(M)]$_2$-NH$_2$ 23. K 109 °C I 105 °C N 60 °C; M 872.1, *m/z* 894.7 $(M+Na^+)$, 872.6 $(M+H^+)$, 559.1 $(M-C_{21}H_{22}NO_3+H^+)$ and 537.0 $(M-C_{21}H_{22}NO_3+H^+)$.

α-Helical peptides

Satisfactory acylation and deprotection traces were obtained for all residues.

Ac-AlaAlaLys(M)AibAlaLys(M)AlaAibAlaLys(M)AlaAla-NH$_2$ 24. mp 196 °C; ν_{max}/cm^{-1} (selected bands) 1659 and 1541; M 1958.7, *m/z* $M+H^++K^+)$, 1004.6 $(M+H^++Na^+)$, 993.6 $(M+2H^+)$ and 985.2 $(M-NH_2^-+H^+)$.

Ac-AlaAlaGlu(M)AibGlu(M)AlaGlu(M)Glu(M)AibGlu(M)-AlaGlu(M)AlaAla-NH$_2$ 25. mp 75 °C; ν_{max}/cm^{-1} (selected bands) 1668 and 1543; M 3095.0, *m/z* 796.7 $(M+4Na^+)$, 701.8 $(M+2H^++2Na^+-C_{21}H_{22}NO_3)$ and 575.6 $(M+5Na^+-C_{21}H_{22}NO_3)$.

β-Sheet peptides

The low solubility of these materials in a range of solvents meant that satisfactory purification was often not possible, and as a result the thermal measurements were carried out on the 'as cleaved' materials. For some peptides it was not possible to acquire ES–MS data; in these cases the identity of the peptides were inferred from the satisfactory acylation and deprotection traces which were obtained during the solid phase synthesis.

H-[ValLys(M)]$_2$-NH$_2$ 26. mp 350 °C (decomp.); ν_{max}/cm^{-1} (selected bands) 1634 and 1550; M 1054.5, *m/z* 1077.3 $(M+Na^+)$, 1055.3 $(M+H^+)$, 547.4 $(M+H^++K^+)$ and 528.3 $(M+2H^+)$.

H-[ValLys(M)]$_3$-NH$_2$ 27. mp 275 °C (decomp.); ν_{max}/cm^{-1} (selected bands) 1633 and 1546; M 1573.2, *m/z* 1573.4 $(M+H^+)$, 1154.3 $[M-Lys(M)+H^+]$, 1055.2 $[M-Lys(M)-Val+H^+]$, 806.8 $(M+H^++K^+)$, 798.7 $(M+H^++Na^+)$ and 787.7 $(M+2H^+)$.

H-[ValLys(M)]$_4$-NH$_2$ 28. mp 340 °C (decomp.); ν_{max}/cm^{-1} (selected bands) 1634 and 1549.

Ac-[ValLys(M)]$_2$-NH$_2$ 29. mp 330 °C (decomp.); ν_{max}/cm^{-1} (selected bands) 1633 and 1550; M 1096.5, *m/z* 1096.3 $(M+H^+)$.

Ac-[ValLys(M)]$_3$-NH$_2$ 30. mp 330 °C (decomp.); ν_{max}/cm^{-1} (selected bands) 1633 and 1547.

Ac-[ValLys(M)]$_4$-NH$_2$ 31. mp 340 °C (decomp.); ν_{max}/cm^{-1} (selected bands) 1634 and 1548.

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