AcOEt/hexane (5/1) [$^{15}N_2$]-**1** (R_f 0.88 in $CHCl_3$/EtOH (10/1)) was eluted first, followed by unreacted aniline (R_f 0.67 in $CHCl_3$/EtOH (10:1)); with AcOEt [$^{15}N_5$]-**2** was eluted (R_f 0.46 in $CHCl_3$/EtOH (10:1)); finally, with $CHCl_3$/EtOH (10/1), the starting material [$^{15}N_4$]-**4** was recovered (R_f 0.24 in $CHCl_3$/EtOH (10:1)). Product: [$^{15}N_5$]-**2** (0.21 g, 49%); mp 213.9°C (decomp); ^{1}HNMR ($CDCl_3$): $\delta = 12.77$ (ddd, $^1J_{N1,H10} = 88.6$, $^3J_{H10,H2} = 13.9$, $^{1h}J_{N9\cdots H10} = 4.4$ Hz; H10), 8.54 (ddd, $^2J_{H8,N9} = 2.4$, $^3J_{H8,N1'} = 7.3$ Hz; H8), 8.51 (m; H2′ and H5′), 8.06 (td, $^4J_{H2,N9} = {}^5J_{H2,H6} = 0.9$ Hz; H2), 7.44 (t; ArH(*m*)), 7.26 (ddd; H6), 7.24 (m; ArH(*o*)), 7.20 (m; ArH(*p*)), 7.08 (ddd, $^4J_{H6,H4} = 1.9$, $^5J_{H4,H8} = 0.7$ Hz; H4), 6.53 (dd, $^3J_{H5,H6} = 3.2$, $^3J_{H5,H4} = 4.3$ Hz; H5); ^{13}CNMR ($CDCl_3$): $\delta = 157.9$ ($^1J = 159.4$, $^3J_{C,H} = 4.0$, $^1J_{C,N} = 4.8$ Hz; C8), 143.9 ($^1J = 166.7$, $^1J_{C,N} = 15.9$ Hz; C2), 141.4 ($^1J = 159.4$, $^3J_{C,N} = 4.8$ Hz; C6), 139.0 ($^1J_{C,N} = 14.8$ Hz; ArC(*i*)), 138.4 ($^1J = 211.1$, $^1J_{C,N} = 12.3$ Hz; C2′ and C5′), 135.9 ($^1J = 166.4$, $^3J_{C,N} = 3.5$ Hz; C4), 130.2 ($^1J = 162.1$, $^3J_{C,N} = 1.9$ Hz; ArC(*m*)), 125.8 ($^1J = 163.8$ Hz; ArC(*p*)), 122.3 ($^1J = 168.7$ Hz; C5), 120.0 ($^2J_{C,N} = 5.2$, $^3J_{C,N} = 8.5$ Hz; C7), 117.4 ($^1J = 155.3$ Hz; ArC(*o*)), 116.9 (C3); ^{15}N NMR ($CDCl_3$): $\delta = -238.1$ ($^{2h}J_{N1-H10\cdots N9} = 8.6$ Hz; N1), −167.7 ($^1J_{N9,N1'} = 11.8$ Hz; N1′), −121.0 ($^{2h}J_{N1-H10\cdots N9} = 8.6$, $^1J_{N9,N1'} = 11.8$ Hz; N9), −64.4 (N3′ and N4′); ^{15}N NMR ($CDCl_3$+D_2O): $\delta = -239.3$ ($^1J_{N1,D} = 13.2$, $^{2h}J_{N1-D\cdots N9} = 8.5$ Hz; N1), −167.3 ($^1J_{N9,N1'} = 12.0$ Hz; N1′), −120.9 ($^1J_{N9,N1'} = 11.9$, $^{2h}J_{N1-D\cdots N9} = 8.5$ Hz; N9), −66.8 (N3′ and N4′); elemental analysis calcd for unlabeled $C_{15}H_{13}N_5$: C 68.42, H 4.98, N 26.60; found: C 68.60, H 4.96, N 26.52.

The N-methyl derivative of [$^{15}N_5$]-**2** in [D_8]THF presents the following ^{15}N NMR signals: $\delta = -254.2$ (N1), −163.6 (N1′), −100.2 (N9) and −63.5 (N3′ and N4′).

Received: September 4, 2000 [Z15758]

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Identification and Isolation of a Receptor for *N*-Methyl Alkylammonium Salts: Molecular Amplification in a Pseudo-peptide Dynamic Combinatorial Library**

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Dynamic combinatorial chemistry[1] (DCC) offers a new strategy for the identification of new host and guest compounds with the potential for catalysis and drug activity.[1, 2] It combines the merits of combinatorial chemistry[3] with molecular evolution, whereby a combinatorial library of candidate molecules is generated by the assembly of building blocks through reversible bonds. As a consequence, all the library members are interconverting through exchange processes to give a product distribution which is under thermody-

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[**] This work was supported by the Biotechnology and Biological Sciences Research Council (UK), Engineering and Physical Sciences Research Council (UK), Ethyl Corporation (UK), and Fundacion Antorchas and Consejo Nacional de Investigaciones Cientficas y Tecnicas (Argentina).

namic control and which may be influenced by template effects. Candidate molecules which bind strongly to templates will become amplified in concentration, while those that exhibit poor binding to the template will decrease in concentration by virtue of the Le Chatelier principle (Figure 1). Here

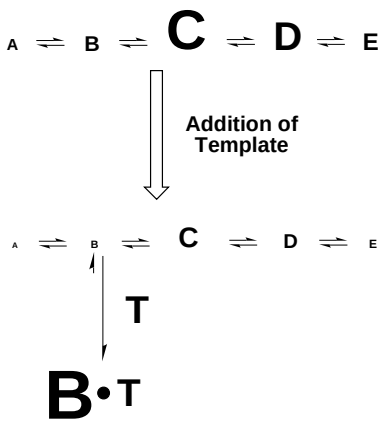
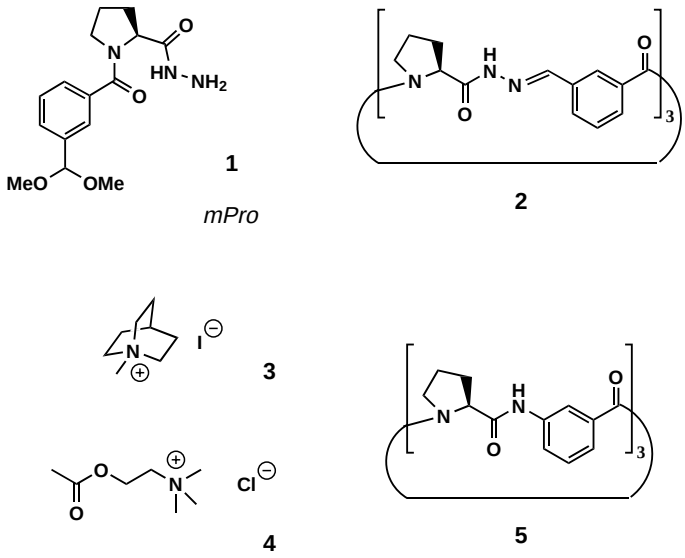


Figure 1. Schematic representation of the interconversion of library members by equilibrium processes and the subsequent product distribution change exerted by a template (**T**). Letter sizes are representative of product concentration.

we report molecular amplification from a dynamic combinatorial library (DCL) of pseudo-peptides of a macrocyclic receptor that binds selectively to acetylcholine and *N*-methyl quinuclidinium salts. The shifts in product distribution we report are to our knowledge the largest yet observed for covalent dynamic combinatorial systems, and serve as proof of the concept that dynamic combinatorial chemistry (DCC) may be used to identify and isolate new molecules from large pools of candidates through noncovalent template effects.

DCLs have been generated noncovalently through metal–ligand exchange[4] and hydrogen-bonding networks,[5] and covalently deploying a range of reversible reactions[1] such as transesterification,[6] disulfide exchange,[7] allylpalladium chemistry,[8] and transimination.[9] However, reported examples of successful templating of covalent DCLs are so far very limited. We have generated libraries of hydrazone-based pseudo-peptides which interconvert by transimination at the hydrazone bond,[10] and have recently reported significant molecular amplification in such a library by complexation of [18]crown-6 to the monomer hydrazinium cation.[11] This effect is general for hydrazinium cations, but we now report specific molecular amplification of a macrocycle in a dynamic combinatorial mixture of hydrazone-based pseudo-peptides.

The DCL formed from building block *mPro* **1** was designed to contain a cyclic structure that would emulate cation binding observed with Kubik's cyclic peptides.[12] Kubik's cyclic peptide **5** binds acetylcholine (ACh) iodide and *N*-methyl quinuclidinium (NMQ) iodide in chloroform with stability constants of 11000 M^{-1} and 42200 M^{-1}, respectively.[12a] Comparison of the *mPro* trimer **2** with receptor **5** reveals very similar structures, with the exception of six additional bonds in our receptor. The expectation was that ACh and NMQ would bind the *mPro* cyclic trimer **2** strongly enough to template its formation leading to molecular amplification.



L-Proline was therefore equipped with the requisite hydrazide and aldehyde groups to form oligomeric pseudo-peptide cyclic hydrazones, with a view to biasing the thermodynamic product distribution to favor a complex between the cyclic trimer and the cation. Treatment of the *mPro* monomer **1** at a concentration of 5 mM in chloroform with one equivalent of trifluoroacetic acid (TFA) affords the complete range of cyclic oligomers from cyclic dimer to cyclic 15mer as kinetic products, the dominant kinetic product being cyclic trimer, according to HPLC and ESI-MS. Hydrazone exchange is catalyzed by TFA such that the reaction mixture, over a period of two days, reaches thermodynamic equilibrium. In the case of the *mPro* homooligomeric library proof-reading[13] and editing led to a thermodynamic mixture, as recorded by HPLC, which comprises mainly cyclic dimer (88%) and cyclic trimer (11%) (Figure 2b). This is an example of thermody-

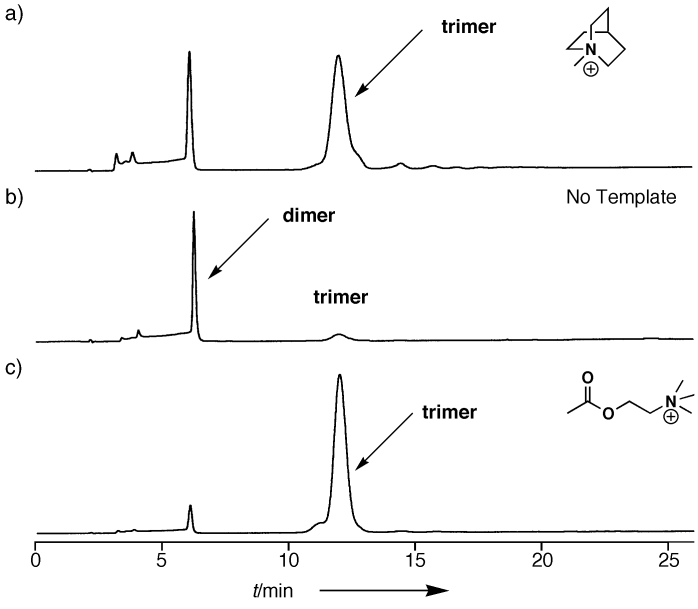


Figure 2. a) HPLC trace for **1** cyclized in the presence of two equivalents of *N*-methyl quinuclidinium iodide after two days. b) Compound **1** cyclized in the absence of template under the same conditions. c) Cyclization in the presence of two equivalents of acetylcholine.

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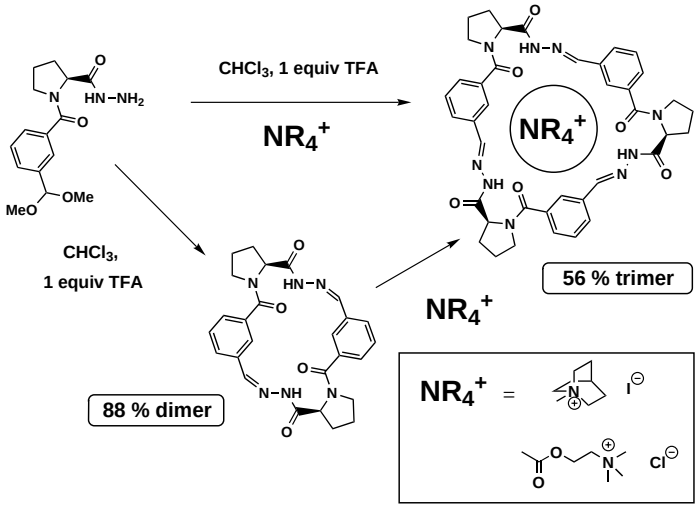
namic control over product distribution in a DCL, whereby a diverse library of kinetic products—more diverse than the 14 cyclic species observed by mass spectrometry because of *cis*/*trans* isomers, conformers, and low-level linear species—thermodynamically evolves to form the more energetically favorable dimer. ^{1}H NOESY analysis of the cyclic dimer in $CDCl_3$ and inspection of models led us to believe that $\pi-\pi$ stacking[14] between the aromatic units of the two monomers may contribute to dimer stability.

Cyclization of **1** in chloroform at 5 mM in the presence of two equivalents of NMQ iodide with one equivalent of TFA reveals a dramatically different library composition strongly biased towards cyclic trimer—cyclic dimer (41%) and cyclic trimer (56%)—with traces of higher oligomers (Figure 2a). This product distribution is attained irrespective of whether introduction of the template occurs before addition of TFA or to the equilibrating mixture containing predominantly dimer (Scheme 1). This confirms that the distribution observed is



Scheme 1. The two routes possible for templating of the product distribution towards cyclic trimer, included intermediate thermodynamic synthesis of cyclic dimer.

representative of a thermodynamically controlled process rather than kinetic trapping of cyclic trimer. Acetylcholine (ACh) chloride exerts an even greater influence on the product distribution, giving under the same conditions 86% trimer, 13% dimer, and insignificant quantities of higher oligomers (Figure 2c). The change in product distribution represents more than 50-fold amplification of cyclic trimer relative to cyclic dimer. As expected, increasing the concentration of template increases the bias towards trimer.

Our first evidence that these observations are the result of specific template effects is mass spectrometry. Electrospray mass spectrometry is a powerful technique for identifying supramolecular complexes,[15] and under some conditions can give estimates of relative stability constants for such adducts.[16] Analysis of the cyclization reactions templated with NMQ and ACh revealed the dimeric ($[M+H]^+ = 487.3$) and trimeric ($[M+H]^+ = 730.4$) cyclic oligomers with traces of the higher oligomers. Significantly, adducts of NMQ and ACh are observed for the trimer only; no such adducts are observed with the dimer (Figure 3).

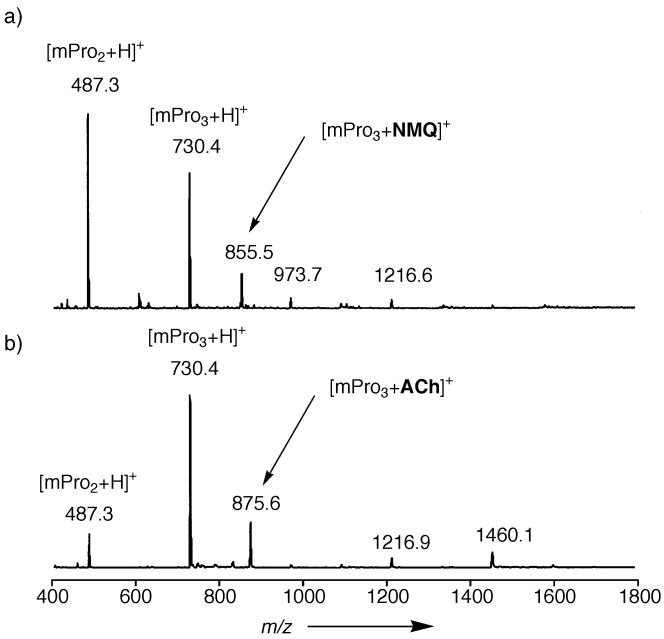


Figure 3. a) Electrospray mass spectrum of the cyclization mixture of **1** in the presence of *N*-methyl quinuclidinium iodide, depicting the trimer–quinuclidinium adduct. b) The mass spectrum for the analogous experiment with acetylcholine.

Pure trimer was readily isolated on a hundred milligram scale by silica gel chromatography of a reaction mixture templated by NMQ. The reaction was washed with water to remove much of the TFA and NMQ template and then evaporated under reduced pressure for purification by chromatography; under the conditions, template and receptor are sufficiently dissociated to allow separation.

The ^{1}H NMR spectrum of template-free trimer in a 95:5 chloroform/methanol mixture—to ensure complete solubility—is complicated by the presence of *cis*/*trans* and conformational isomers of the trimer (Figure 4b). Inspection of the α-proton region of the spectrum reveals one major conformer ($\delta = 4.74$) and a range of α-proton signals between $\delta = 4.5$ and 6.0. That all of these conformers for the trimer are interconverting is clear from the NOESY spectrum which shows exchange peaks between all the α-proton signals. The α-proton signals at $\delta = 5.63$ and 5.74 have shifts comparable to that observed for the dimer, suggesting that some of the trimer conformers may be folded by $\pi-\pi$ stacking in a similar way to cyclic dimer.

The addition of a template to cyclic trimer dramatically changes the ^{1}H NMR spectrum of each component: adding one equivalent of NMQ or ACh generates a simpler trimer spectrum dominated by a single conformer (Figure 4),[17] accompanied by downfield shifts for the *N*-methyl protons by 0.15 ppm and 0.27 ppm for ACh and NMQ, respectively. The chirality of the receptor induces significant changes in the ^{1}HNMR signals for the CH_2 groups α to the ammonium center: the two protons are now nonequivalent (Figure 4). In the case of ACh the appearance of the signals for -CH_2**a**- and -CH_2**b**- and the observed *gem*, *cis*, and *trans* coupling constants for -CH_2**b**- ($J = 15.7$, 2.9, and 7.5 Hz, respectively, Figure 5) led to the interpretation that acetylcholine is bound

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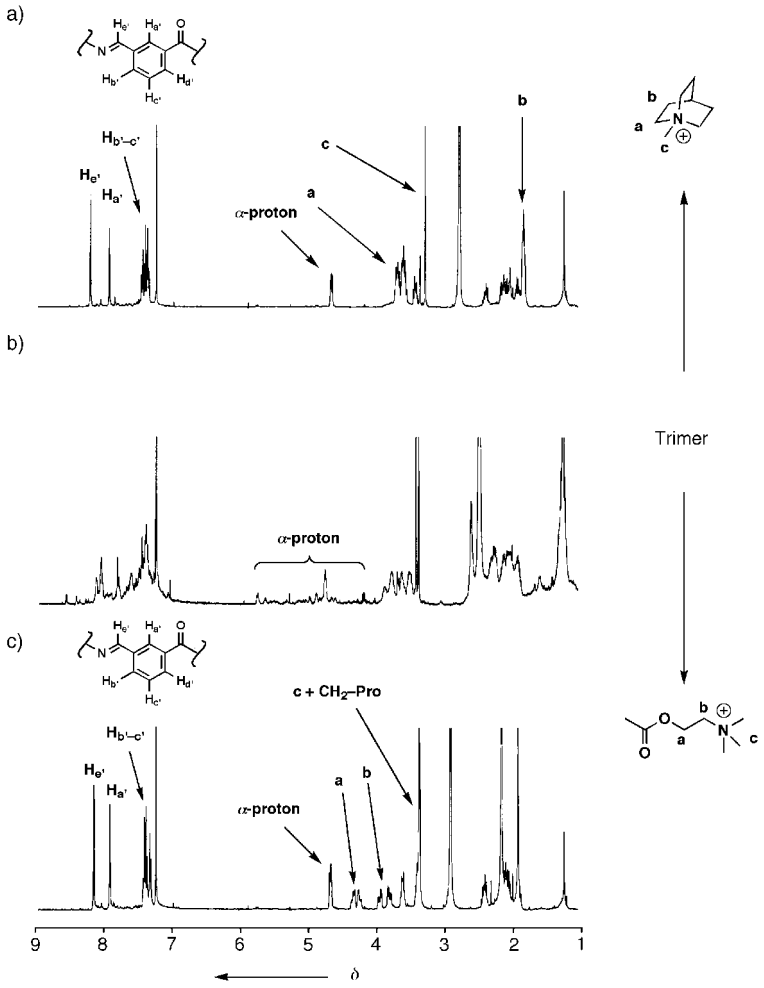

Figure 4. a) Partial 400 MHz ^{1}H NMR spectrum for cyclic trimer with one equivalent of *N*-methyl quinuclidinium salt, showing assignments for the trimer aromatic signals, trimer α-proton, and selected template protons. b) Partial ^{1}H NMR spectrum for cyclic trimer of **5**, highlighting the complex array of α-proton signals arising from multiple conformers. c) The ^{1}H NMR spectrum for cyclic trimer in the presence of one equivalent of acetylcholine, including some selected assignments for the template and host.

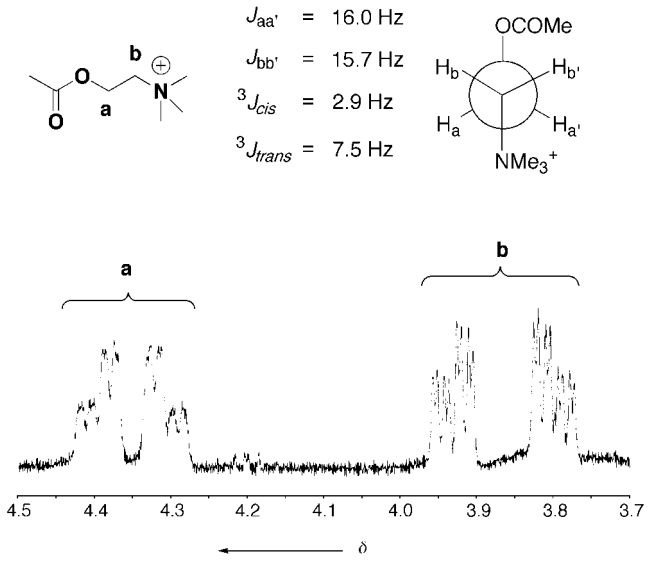

Figure 5. Acetylcholine CH_2**a**CH_2**b** resonances in the 500 MHz ^{1}H NMR spectrum of the acetylcholine-cyclic trimer adduct, with derived binding conformation of the guest. Stereospecific assignments of H_a versus $H_{a'}$ and H_b versus $H_{b'}$ are unknown.

in the *trans* conformation.[18] Control experiments of 1:1 mixtures of templates with cyclic dimer afforded ^{1}H NMR spectra which show none of the changes in chemical shifts or splitting of CH_2 signals observed with cyclic trimer. In conjunction with an absence of a dimer·template adduct in the mass spectra, this represents good evidence that only the cyclic trimer binds to the ammonium salt templates. Significant binding to higher oligomers is excluded by the observation that they are not amplified by the addition of templates.

Binding constants for the two templates to the cyclic trimer in a $CDCl_3/CD_3OD$ mixture (95:5) were estimated as 230 M^{-1} and 150 M^{-1} for ACh and NMQ, respectively, by monitoring the changes in chemical shift for the *N*-methyl groups during ^{1}HNMR titrations. Good fits with uncertainties of less than 5% were obtained on the basis of simple 1:1 equilibria. These relative binding constants match the templating response, the shift towards cyclic trimer being 1.5-fold greater for ACh than for NMQ. However, the conditions under which titrations were performed do not entirely reflect those of the cyclization reaction (100% chloroform with 1 equiv TFA). It is reasonable to believe that the presence of 5% methanol could give weaker binding than would be operating under the reaction conditions. Kubik's cyclic peptide **5** has a reversed preference for the acetylcholine and *N*-methyl quinuclidinium salts; it happens that our receptor is more selective for acetylcholine.

The most direct evidence for supramolecular complexation between cyclic *mPro* trimer **2** and NMQ comes from ^{1}H NOESY analysis of a 1:1 mixture in $CDCl_3/CD_3OD$ (95:5) at 40 °C. Despite elevated temperatures the binding between the trimer and NMQ guest is strong enough for NOEs to be observed between the *N*-methyl, CH_2**a** and CH_2**b** protons of NMQ with Ar-$\mathbf{H_{a'}}$ of the cyclic trimer. The strongest NOE signals are for the CH_2**a** and *N*-methyl groups suggesting that the ammonium center is directed at the cavity of the trimer. The NOESY spectrum acquired of a 1:1 mixture of ACh and cyclic trimer under the same conditions as for quinuclidinium also exhibits a small NOE between the acetylcholine NMe_3 protons and cyclic trimer.

The NOESY spectra for the trimer in the presence of template are dominated by exchange peaks between the dominant trimer conformer and the minor conformers that are present in very low concentrations; this has prevented elucidation of a binding conformation for the trimer. Identification of the noncovalent interactions responsible for the binding observed is therefore speculative. In the trimer·template complexes the *N*-methyl groups and α-CH_2 protons of ACh and NMQ experience downfield shifts which are characteristic of hydrogen bonding, whereas cation–π complexation of cations in cavities lined with aromatic subunits generally causes an upfield shift of the guest protons. The positive charge of alkylammonium cations is somewhat dispersed rather than resident as a point charge on the

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ammonium center,[19] so methylene protons adjacent to ammonium centers are slightly acidic, giving rise to hydrogen bonding capabilities. We believe that C–H···O hydrogen bonding[20] is likely to occur between the CH_2 groups of the ammonium salts and the amide C=O oxygen atoms, and is a driving force for templating and molecular amplification in our system. This binding rationale contrasts with that applied to other synthetic acetylcholine receptors where cation–π interactions are invoked.

In conclusion, we have reported proof-reading to form cyclic dimer from a diverse kinetic combinatorial library of hydrazone-based cyclic pseudo-peptide oligomers. Using *N*-methylammonium salts acetylcholine chloride and quinuclidinium iodide we have been able to thermodynamically bias the product distribution of the dynamic combinatorial library towards a cyclic trimer. Binding between the cations and cyclic trimer has been unequivocally proven by mass spectrometry and ^{1}HNMR spectroscopy. To date this is the most significant example of templating in a covalent dynamic combinatorial library, facilitating the identification and isolation of a specific receptor. Extension of this approach to more diverse dynamic combinatorial libraries may afford highly effective receptors for acetylcholine and quaternary ammonium compounds as mimics for a wide range of biological binding sites. The concept of thermodynamic control of product distributions in DCLs by template effects has been proven and sets the scene for the identification of new, more efficient and selective receptors and enzyme mimics.

Experimental Section[21]

mPro **1**: 1) L-Proline methyl ester hydrochloride (0.59 g, 3.56 mmol) and 3-carboxybenzaldehyde dimethoxyacetal (0.70 g, 3.57 mmol) were dissolved in dry CH_2Cl_2 (20 mL) with dry Et_3N (1.0 mL, 7.17 mmol) under Ar (g) and the solution was cooled to 0 °C on an ice bath. To this solution were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.68 g, 3.57 mmol) and 4-dimethylaminopyridine (DMAP) (25 mg, 0.20 mmol) and the solution stirred at 0 °C for 1 h before being allowed to warm to room temperature and stirred overnight. The work-up involved the addition of CH_2Cl_2 (120 mL) and washing with 3×100 mL portions of H_2O. The organic portion was dried ($MgSO_4$) and the solvent removed in vacuo to afford a yellow oil. This oil was subjected to silica gel chromatography (EtOAc/hexane (8/2)) to afford the target compound as a colorless oil (0.76 g, 69 %): $R_f = 0.46$ (EtOAc/hexane (8/2)); UV, I_2. 2) This oil (0.74 g, 2.41 mmol) was dissolved in MeOH (25 mL) and treated with hydrazine monohydrate (1.2 mL, 24.7 mmol) at room temperature for 20 h. Evaporation of the solvent under reduced pressure afforded a yellow oil which after silica gel column chromatography (CH_2Cl_2/MeOH (90/10)) yielded the target monomer **5** as a white foamy oil (0.57 g, 77 %): $R_f = 0.48$ (CH_2Cl_2/MeOH (90/10)); UV, I_2; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.31$ (s, 1H; NH), 7.62 (s, 1H; Ar-H), 7.53–7.48 (m, 2H; Ar-H), 7.40 (t, $^3J(H,H) = 7.6$ Hz, 1H; Ar-H), 5.39 (s, 1H; C*H*(OMe)$_2$), 4.67 (m, 1H; α-H), 3.94 (s, 2H; NHN*H*$_2$), 3.58 (m, 1H; Pro-C*H*$_a$H$_b$), 3.47 (m, 1H; Pro-CH$_a$*CH*$_b$), 3.31 (s, 6H; CH(O*Me*)$_2$), 2.37 (m, 1H; Pro-CH$_2$), 2.07 (m, 2H; Pro-CH$_2$), 1.83 (m, 1H; Pro-CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta =$ 172.0, 170.7, 138.5, 135.9, 128.7, 128.3, 127.3, 125.6, 102.4, 58.6, 52.7, 50.4, 27.8, 25.5; ESI: *m*/*z*: 308.4 $[M+H]^+$, 276.3 $[M-OMe]^+$, 244.4 $[M-2OMe]^+$; HRMS: $C_{15}H_{21}N_3O_4$ $[M+Na]^+$ requires 330.1430, found 330.1446.

Cyclization reactions were carried out in freshly distilled $CHCl_3$ at 5 mM with one equivalent of TFA with respect to monomer. Cyclizations and templated reactions were analyzed by ESI-MS and HPLC. Electrospray mass spectra were recorded on a Micromass Quattro-LC triple quadrupole apparatus fitted with a z-spray source. The electrospray source was heated to 100 °C and the sampling cone voltage (V_c) was 30 V. Samples were introduced into the mass spectrometer source without work-up with an LC pump (Shimadzu LC-9A) at a rate of 4 μL min^{-1} of MeCN/H_2O (1/1). Calibration was performed by using protonated horse myoglobin. Scanning was performed from *m*/*z* 200 to 2200 in 6 s and several scans were summed to obtain the final spectrum which was processed using MassLynx V3.0 software.

HPLC analysis was performed on a Hewlett-Packard 1050 instrument using reversed phase conditions of H_2O:MeCN gradients with a 15 cm × 4.6 mm i.d. 3 μm particle size, Supelco ABZ$^+$plus C16 alkylamide column. Data were analyzed by using HP ChemStation. All NMR spectroscopy was performed on Bruker DRX 400 or DPX 500 instruments and chemical shifts are quoted in parts per million with respect to TMS.

Received: September 29, 2000 [Z15878]

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[21] We thank Dr. Sally-Ann Poulsen for the initial synthesis of the *mPro* **1** monomer.

Synthesis of a Conjugated Macromolecular Initiator for Nitroxide-Mediated Free Radical Polymerization**

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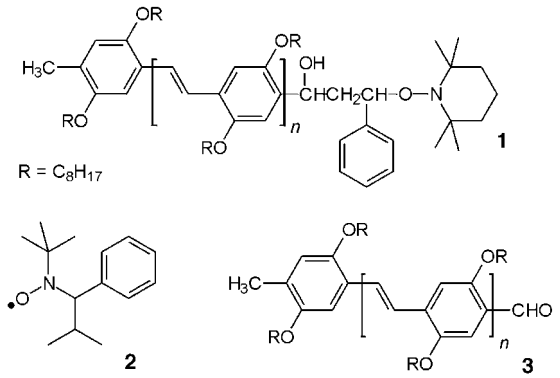
Recent years have seen the increased use of organic materials for opto – electronic applications, such as light-emitting diodes (LEDs) and photovoltaic cells. Such applications require a combination of material properties, for example, good conductivities for holes as well as for electrons, or both electron-donating and electron-accepting capabilities. Since this combination is not likely to be found in a single component, a range of multicomponent systems have been evaluated for use in devices and some have clearly shown beneficial effects.[1] However, simple mixtures containing a polymer component may exhibit macrophase separation. Macrophase separation occurs on a length scale of micrometers (or coarser), which is too large for optimum performance. This is especially true when essential processes rely on the diffusion of excited species to the interface between the components. The diffusion length of a neutral exciton, for example, is of the order of a few nanometers.[2] Hence, in a simple blend, most of the excitons will be lost to some decay channel before having a chance to interact with the second component.

When it comes to the combination of desirable properties in a single chemical compound, block copolymers may be the materials of choice. They might show the phenomenon of microphase separation (if the product of molecular weight (N) and interaction parameter (χ) is large enough) into various ordered structures on a nanometer scale, while the blocks of one macromolecule can have different functionalities.[3] These properties comply well with the requirements for performance enhancements of optoelectronic devices, and our research therefore focuses on the preparation and evaluation of appropriately functionalized block copolymers.

Our goal is to obtain block copolymers suitable for photonic applications such as LEDs or photovoltaic cells. We chose one of the blocks as poly(2,5-dioctyloxy-1,4-phenylene vinylene) (PPV), a polymer frequently used in opto – electronic applications serving as the hole-transporting and luminescent material in an LED or as the light absorber and electron donor in a photovoltaic cell.[1] The second block should contain the complementary functionality required for the specific application: an electron-conducting material to achieve balanced charge transport in LEDs (e.g. an oxadiazole) or an electron acceptor (e.g. C_{60}) for photovoltaic cells. To achieve microphase separation, the second block should also be incompatible (high value of the interaction parameter) with the PPV block.

Recently, we published our approach to synthesizing this kind of molecule, which involves the attachment of an alkoxyamine to the PPV block.[4] This yields a macroinitiator **1**, which is suitable for nitroxide-mediated free radical



polymerization (NMRP), one of several controlled radical polymerization techniques.[5] The nitroxide we used was the commercially available 2,2,6,6-tetramethylpiperidinyl-*N*-oxyl (TEMPO). While a number of publications on conjugated rod – coil block copolymers have appeared in the literature,[6] only a few deal with the use of controlled radical polymerization techniques to construct the coil block.[7] These rather new methods allow the synthesis of block copolymers and tolerate a wide range of substituents.

The aim of our work, controlling optoelectronic properties by synthesizing appropriate block copolymers, can be achieved by varying two parameters: the length of the blocks (degree of polymerization N) or their degree of incompatibility (described by the χ-parameter).[8] The control over the block length is achieved by using a "living" polymerization method (the NMRP method), in which the block length increases linearly with polymerization time. One of the major drawbacks of the use of TEMPO in this controlled radical polymerization is that control can only be established for

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[**] The financial support of the EC (TMR scholarship for U.S.) and the Dutch Research Foundation, Physics Division (NWO-FOM) is gratefully acknowledged. G. Alberda van Ekenstein is acknowledged for his contribution to the thermal analysis.

Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

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