Synthesis of Components for the Generation of Constitutional Dynamic Analogues of Nucleic Acids

by **David T. Hickman**[1]), **Nampally Sreenivasachary**[1]), and **Jean-Marie Lehn***

ISIS-ULP-CNRS UMR 7006, 8, Allée Gaspard Monge, BP 70028, F-67083 Strasbourg Cedex (phone: +33(0)390245145; fax: +33(0)390245140; e-mail: lehn@isis.u-strasbg.fr)

The introduction of dynamic covalent polymers, in which the monomer units are linked by reversible covalent bonds and can undergo component exchange, opens up new possibilities for the generation of functional materials. Extending this approach to the generation of dynamic biopolymers in aqueous media, which are able to adapt constitution (sequence, length) to external factors (*e.g.*, environment, medium, template), would provide an alternative approach to the *de novo* design of functional dynamic bio-macromolecules. As a first step towards this goal, various mono- and bifunctionalised (hetero- and homotopic) nucleic acid-derived building blocks of type **I**–**X** have been synthesised for the generation of dynamic main-chain and side-chain reversible nucleic acid analogues. Hydrazide- and/or acetal (protected carbonyl)-functionalised components were selected, which differ in terms of flexibility, length, net formal charge, and hydrazide/acetal substituents, in order to explore how such factors may affect the properties (structure, solubility, molecular recognition features) of the polymer products that may be generated by polycondensation.

1. Introduction. – Constitutional dynamic chemistry (CDC) implements reversibility of connections between components in order to generate both molecular and supramolecular systems that are responsive to their environment [1]. Whereas the latter are dynamic by nature, applying this central feature of CDC to the generation of *molecular* diversity requires the operation of reversible covalent reactions to produce the constituents resulting from all possible combination of components, and provides the basis of dynamic combinatorial chemistry (DCC) [2]. Since its inception, DCC has been implemented as a useful approach for the rapid generation and identification of small molecules or small supramolecular assemblies capable of interacting with a target molecule [2], in particular of biological type [3]. However, CDC has also great potential as means for generating dynamic materials [1][2a]. Thus, we have recently become interested in its application within the field of polymer chemistry to produce dynamic polymers, termed *dynamers* [4] in which the monomer units are linked *via* reversible covalent bonds and can undergo component exchange under controlled conditions [5]. We were interested in extending the scope of such *dynamers* to the generation of constitutional dynamic biopolymers in aqueous media, related to nucleic acids [6], peptides [7], and carbohydrates [8]. To these ends, components are required that bear functional groups capable of engaging into reversible covalent reactions. Herein, we describe the synthesis of a range of building blocks as analogues of nucleic

[1]) Present address: *AC Immune SA*, PSE-B, EPFL, CH-1015 Lausanne.

acid components for the generation of both main-chain- and side-chain-reversible polymeric analogues of nucleic acid strands (*Fig. 1*).

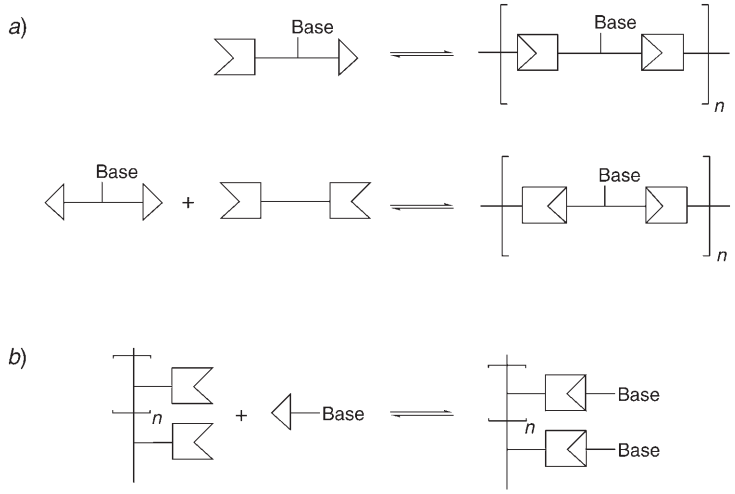


Fig. 1. *Schematic representation of reversible covalent bond formation* a) *between bifunctional hetero- or homotopic nucleobase derivatives to give dynamic main-chain polymers, or* b) *between side-chain-functionalised polymer with monofunctionalised nucleobase derivatives to give dynamic side-chain polymers*

Whilst polymers can potentially cover enormous spatial as well as functional diversity, the *de novo* design of abiotic polymers capable of molecular recognition or catalysis in aqueous media remains a major challenge [9]. We have sought to develop polymeric analogues of DNA which maintain the recognition features of the native nucleobases but contain a reversible covalent bond in the main chain or side chain [10]. The application of DCC to polymers potentially gives rise to vast libraries of polymeric sequences differing both in length and in sequence. Such dynamic polymers, formed under thermodynamic control, may be capable of undergoing self-assembly, component selection, and possibly constituent amplification under the driving force of folding into stable secondary or tertiary structures [11], of substrate binding [6b] or of other effects, like metal ion coordination [12a,b] or formation of an organized phase [12c]. Furthermore, addition of a template such as complementary ssDNA, a target protein or a transition-state analogue may enable the amplification of a single or a small number of sequences from the polymeric library [4a].

2. Results and Discussion. – A range of bifunctionalised nucleotide analogues containing hetero- and homotopic monomer units were synthesised for reversible main-chain polymerisation. Likewise, several monofunctionalised nucleic acid components have also been prepared, which may be used to decorate the side chain of polymers or of core groups of various shapes in a reversible fashion.

Several reversible reactions have been exploited for the generation of dynamic combinatorial libraries in aqueous media including imines [3a,f–i], acylhydrazones

[3c–e][13], disulphides [3b][13], thioesters [14], *Michael* addition of thiols [15], alkene metathesis [16], and enzyme-catalyzed aldol formation [17].

Acylhydrazones are particularly well suited for DCC. Acylhydrazone formation can occur rapidly and in high yield in aqueous solution depending on the nature of the substituents in proximity to the carbonyl/aldehyde functionality, and exchange can be fast at pH 5 or even at physiological pH but is essentially stopped at pH > 9 [18c][19]. For the purpose of generating reversible analogues of nucleic acid strands, the acylhydrazone bond is also appealing as it presents both an amide/peptide bond, which has been used widely as a phosphodiester surrogate in modified oligonucleotides [20], and a reversible imine unit [4b]. We thus chose to select monomeric components bearing hydrazide and aldehyde sites as complementary functional groups.

Attention has been paid here to the synthesis of components which differ in terms of flexibility, length, net formal charge, and the types of functional groups adjacent to the hydrazide or to masked aldehyde groups (*Fig. 2*). It was envisaged that such factors would affect the extent of condensation, solubility, and molecular recognition properties of the polymers. In addition, building blocks were selected such that the recognition groups be distant from the aldehyde or hydrazide (or amine) moieties, so as to favour the formation of isoenergetic [2a] libraries. Finally, component structures were designed such that after acylhydrazone formation, the polymer products would resemble other analogues of DNA strands which are known to show high binding affinity to complementary ssDNA [21–23].

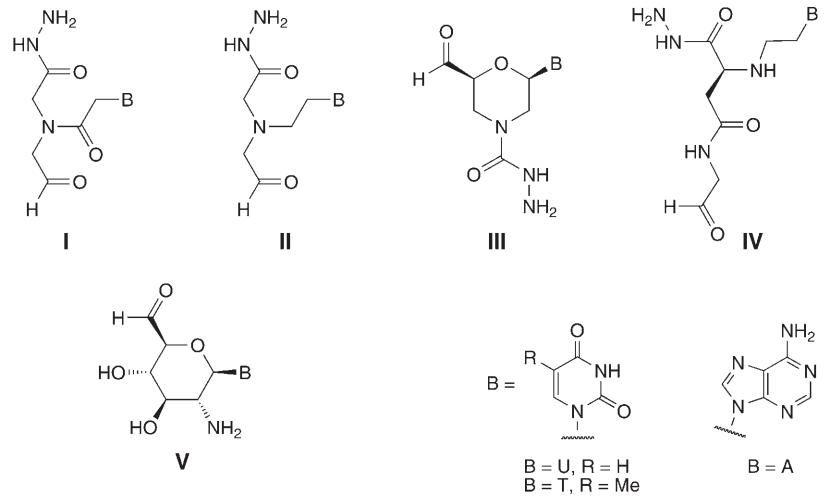


Fig. 2. *Overview of bifunctional heterotopic nucleotide analogues* **I**–**V**

2.1. *Hetero-Bifunctionalised Nucleotide Analogues.* A poly(acylhydrazone) generated by polycondensation of a monomer of type **I** (*Fig. 2*) is structurally similar to PNA [21] but contains in addition reversible acylhydrazone bonds instead of the amide bond. Monomer **I** was also selected as a target because a monomer unit should not readily undergo intramolecular cyclisation and was synthetically appealing, as it contains the same nucleobase-carboxylic acid moiety as found in PNA, for which synthetic protocols

are well-established [24]. To ensure high water solubility of the polymer product, we decided to investigate also nucleotide analogues incorporating a positive charge. Indeed, this could increase binding affinity for the DNA polyanion *via* coulombic attraction. We thus selected monomers of type **II** (*Fig. 2*) which are identical to the acylhydrazone linked PNA monomers except that the C=O group has been replaced by a CH_2 group. The resulting tertiary amine should be substantially protonated in the pH range 5.0–7.0 [25]. In addition, it has been shown that an aldehyde group α to the tertiary amine of piperidine reacts readily with acetylhydrazide, albeit slower than the α-oxy or α-amido aldehydes [19b].

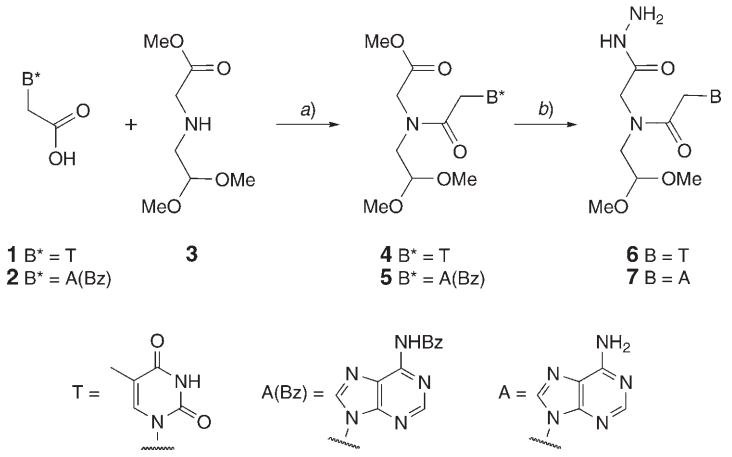
Results from our laboratory have revealed that α-oxy aldehydes react very fast and to a high percent of formation with hydrazides, and undergo rapid exchange upon addition of a second hydrazide [19b]. We thus envisaged that nucleotide analogues of type **III**, derived from morpholine (*Fig. 2*), may likewise undergo condensation and exchange. Furthermore, such constrained monomers should not be susceptible to intramolecular cyclisation and are structurally related to the monomeric repeat units of modified oligonucleotides known to hybridise strongly to complementary ssDNA [22]. Monomer units of type **IV** (*Fig. 2*) have a more flexible backbone and contain an α-amido aldehyde functionality for acylhydrazone formation and exchange, as well as a nucleobase linked through a secondary amine group so as to favour aqueous solubility.

Length-specific oligomerisation of nucleotide analogues bearing amine and aldehyde functionalities has been shown to be catalysed by complementary ssDNA upon addition of sodium cyanoborohydride ($NaCNBH_3$) [26]. We were, however, intrigued by the possibility of using monomer units which could form polyimines in the absence of a template. Previous studies in our laboratory revealed that aldehydes with an α-OH group form particularly stable imines, presumably through H-bond stabilisation of the intermediate hemi-aminal [27]. Monomers of type **V** (*Fig. 2*), which are derived from Glucopyranosyl Nucleic Acids (GNA) [23], satisfy this criterion. In addition, such monomers cannot undergo cyclisation due to structural constraints, and, furthermore, GNA oligomers hybridise with complementary ssDNA with an average increase in T_m of $+1.0^\circ$ per modification compared with isosequential ssDNA [23].

Toward the synthesis of type **I** monomers (*Fig. 2*), nucleobase acids **1** and **2** were synthesised as described in the literature [24b,c], while amine **3** was obtained by reductive amination of glyoxal dimethyl acetal with glycine methyl ester hydrochloride [28]. Coupling reactions of these nucleobase acids **1** and **2** with amine **3** were effected using diisopropylcarbodiimide/1-hydroxybenzotriazole (HOBt) in DMF to give the corresponding ester derivatives **4** and **5**, respectively (*Scheme 1*). Treatment of ester **4** with 5 equiv. $NH_2NH_2 \cdot H_2O$ in EtOH resulted in precipitation of the corresponding hydrazide **6**. Similarly, ester **5** reacted with 5 equiv. $NH_2NH_2 \cdot H_2O$ in EtOH with concomitant removal of the N^6-benzoyl (Bz) protecting group to give hydrazide **7**. Due to their potential to undergo spontaneous polymerization, all bifunctionalised monomers were synthesised with acid-sensitive protecting groups such that subsequent deprotection could be performed *in situ* using HCl without introducing undesirable side-products into the reaction medium.

For the synthesis of monomers of type **II** (*Fig. 2*), aldehyde **8** was synthesised according to a known procedure [29]. The ester **9** was then obtained by reductive

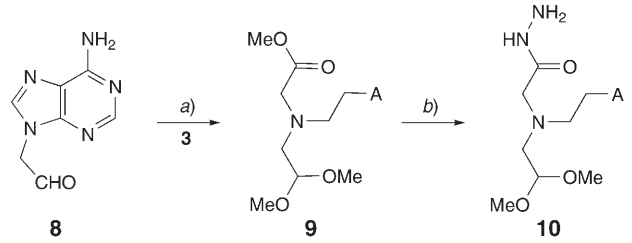
Scheme 1. *Synthesis of the Protected Bifunctional Thyminyl and Adeninyl Monomers* **6** *and* **7**



a) Diisopropylcarbodiimide, 1-hydroxybenzotriazole (HOBt), DMF, r.t. *b*) $NH_2NH_2 \cdot H_2O$, EtOH, r.t.

amination of aldehyde **8** with amine **3** using $NaCNBH_3$ and 4 equiv. of AcOH as catalyst. Treatment of ester **9** with $NH_2NH_2 \cdot H_2O$ gave the corresponding hydrazide **10** (*Scheme 2*).

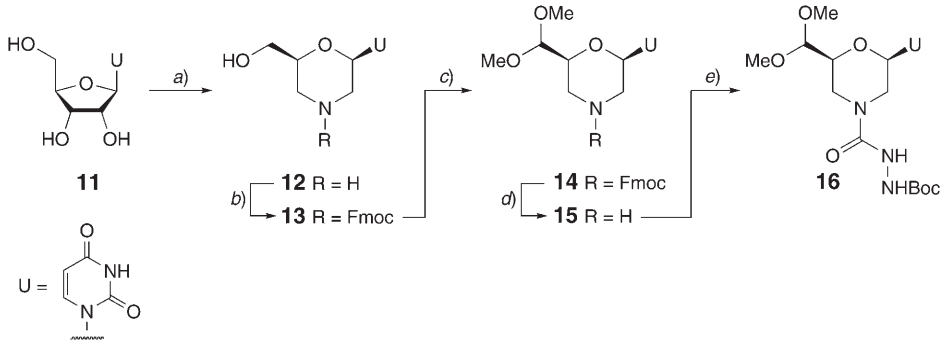
Scheme 2. *Synthesis of the Protected Bifunctional Adeninyl Monomer* **10**



a) $NaCNBH_3$, MeOH, AcOH, r.t. *b*) $NH_2NH_2 \cdot H_2O$, EtOH, r.t.

Next, we turned our attention to the synthesis of nucleotide analogues of type **III** (*Fig. 2*). Periodate cleavage of the 1′,2′-diol group of uridine **11**, followed by reductive amination using ammonium tetraborate, gave the morpholine-derived nucleoside **12** [30]. This amine **12** was protected with the (9*H*-fluoren-9-yl)methoxycarbonyl (Fmoc) group upon reaction with Fmoc chloride to give the alcohol **13** in 93% yield (*Scheme 3*). After testing several oxidation methods, we found that the best yield for the oxidation of alcohol **13** was obtained using the *Dess–Martin* periodinane reagent to give the corresponding aldehyde. This aldehyde was then immediately protected as its dimethyl acetal using trimethyl orthoformate and TsOH to give acetal **14**. Deprotection of the Fmoc group from **14** was carried out using a 5% (*v*/*v*) solution of piperidine in THF, resulting in amine **15**. Formation of the protected semicarbazide was achieved by firstly reacting *tert*-butyl carbazate (NH_2NHCOO^tBu) with *N*,*N*′-carbonyldiimidazole

Scheme 3. *Synthesis of the Protected Bifunctional Uridinyl Monomer* **16**

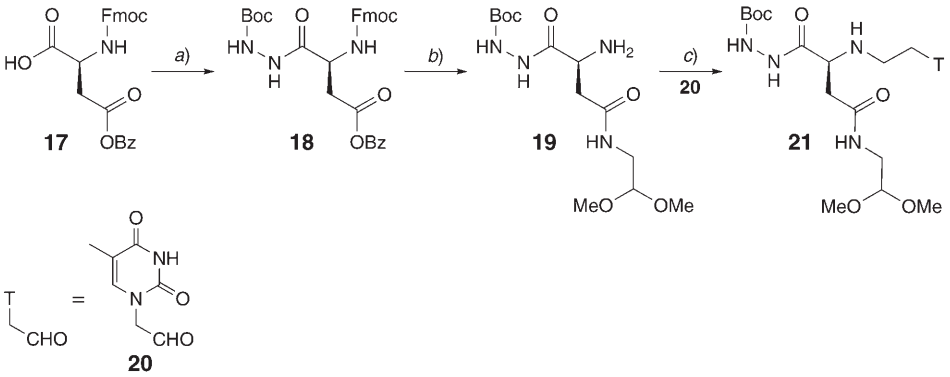


a) $NaIO_4$, ammonium tetraborate, r.t. *b*) (9*H*-fluoren-9-yl)methoxycarbonyl (Fmoc) chloride, EtN(i-Pr)$_2$ (DIPEA), THF, 5° → r.t. *c*) *Dess–Martin* periodinane, CH_2Cl_2; then trimethyl orthoformate, MeOH, TsOH, r.t. *d*) 5% (*v*/*v*) piperidine in THF, r.t. *e*) *N*,*N*′-carbonyldiimidazole, *tert*-butyl carbazate (NH_2NHCOO^tBu), DMF, r.t.; then **15**, DMF, r.t.

in DMF for 5 h, followed by coupling with amine **15** to give acetal **16** in 80% yield (*Scheme 3*).

A short and convenient synthetic route was developed toward monomer **IV** (*Fig. 2*) starting from aspartic acid derivative **17**. Coupling of **17** with NH_2NHCOO^tBu using diisopropylcarbodiimide and HOBt gave ester **18** (*Scheme 4*). Treatment of **18** with 2 equiv. of aminoacetaldehyde dimethyl acetal for 48 h in THF resulted in the simultaneous displacement of the benzyl ester and deprotection of the Fmoc protecting group to give the amino derivative **19** in 70% yield. Finally, **19** was reacted with thymine aldehyde **20** [29] under reductive amination conditions to give the thymynylamino derivative **21**.

Scheme 4. *Synthesis of the Hydrazido-Protected Thyminyl Monomer* **21**

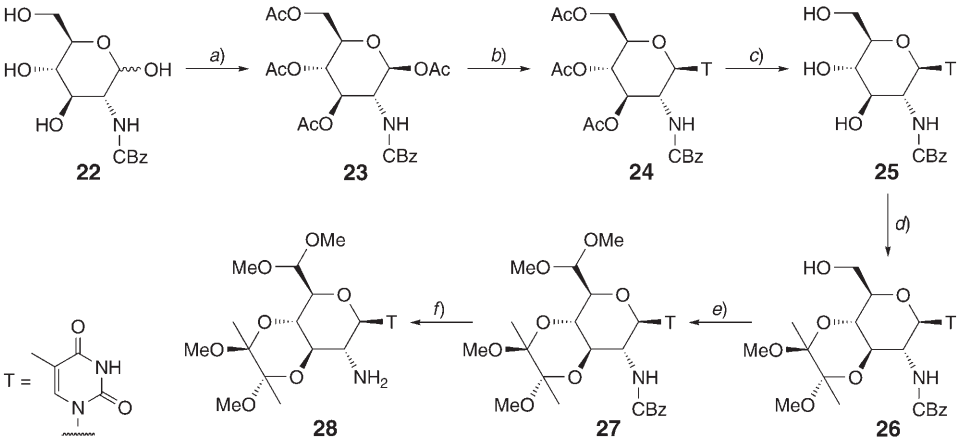


a) Diisopropylcarbodiimide, NH_2NHCOO^tBu, HOBt, r.t. *b*) Aminoacetaldehyde dimethyl acetal, THF, r.t. *c*) **20**, $NaCNBH_3$, AcOH, MeOH, r.t.

To synthesise the monomer unit containing amine and aldehyde functionality such as **V** (*Fig. 2*) required a synthetic route different from that used to synthesise the

monomers of GNA, since the latter retained protecting groups which were incompatible with our needs for mild *in situ* deprotection. The synthesis starts from the (benzyloxy)carbonyl (CBz)-protected D-glucosamine derivative **22** (*Scheme 5*) [31]. Acetylation of **22** using Ac_2O in pyridine gave the desired β-anomer **23** after crystallisation. Insertion of thymine at the anomeric position under *Vorbrüggen* conditions gave thymine derivative **24** in 80% yield, which was deacetylated with Et_3N in $MeOH/H_2O$ to give the triol **25** in 90% yield. The *cis*,*trans* secondary diols of **25** could be selectively protected by reaction with butane-2,3-dione and trimethyl orthoformate using an acid catalyst to give **26** in 72% yield (*Scheme 5*). Oxidation of **26** using trichloroisocyanuric acid and 2,2,6,6-tetramethylpiperidinooxy (TEMPO) gave the corresponding aldehyde, which was directly protected as its dimethyl acetal using trimethyl orthoformate to give acetal **27**. Transfer hydrogenation using cyclohexene removed the CBz protecting group on **27** to give the amino derivative **28** in 69% yield.

Scheme 5. *Synthesis of the Aldehyde-Protected GNA-Like Bifunctional Monomer* **28**



a) Ac_2O, pyridine, $0° \rightarrow$ r.t. *b*) Thymine, *N*,*O*-bis[(trimethylsilyl)acetamide], MeCN, 20° then **23**, trimethylsilyl trifluoromethanesulfonate, 80°. *c*) Et_3N, MeOH, H_2O, r.t. *d*) Butane-2,3-dione, (+)-camphor-10-sulfonic acid monohydrate, trimethyl orthoformate, MeOH, reflux. *e*) Trichloroisocyanuric acid, 2,2,6,6-tetramethylpiperidinooxy (TEMPO), CH_2Cl_2, r.t. then trimethyl orthoformate, (+)-camphor-10-sulfonic acid monohydrate, MeOH, r.t. *f*) Cyclohexene, Pd/C, EtOH, reflux.

2.2. *Self-Condensation of Hetero-Bifunctional Nucleotide Analogues.* The ability of hydrazides **6** and **7** to oligomerise upon acetal deprotection was evaluated next. The dimethyl acetal groups of **6** and **7** (200 mM each) were hydrolysed *in situ* with 0.3M and 0.5M DCl, respectively, in D_2O, so that the deprotection could be monitored by ^{1}H-NMR. During 48 h, acetal deprotection was found to be complete whereupon the solution was immediately diluted tenfold to give 20 mM solutions in acetate buffer with final pD values of 3.4, 5.0, and 7.0. Both MALDI- and ESI-MS spectra of these solutions showed peaks at *m*/*z* 863 and 1144 corresponding to linear trimeric (pD 3.4 only) and tetrameric oligomers, respectively.

To better understand why longer oligomers or polymers were not formed, the condensation of equimolar amounts of hydrazide **7** with different aldehyde hydrates **8**, **51**, and **52**, was evaluated as a function of time using ^{1}H-NMR spectroscopy (*Fig. 3*).

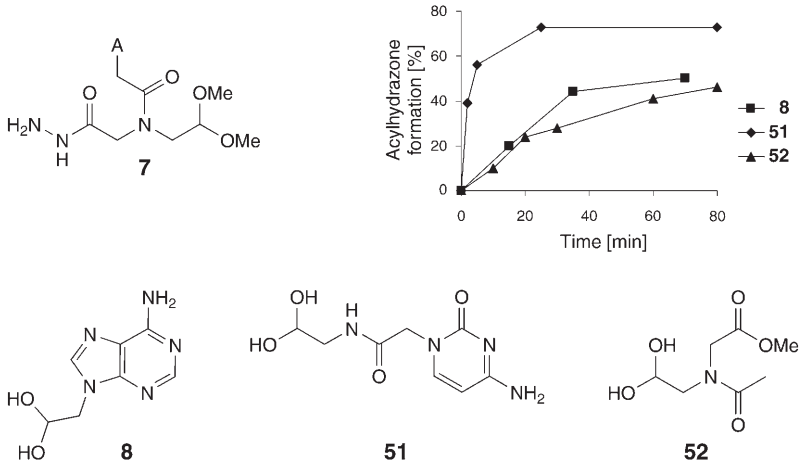


Fig. 3. *Condensation of hydrazide* **7** *with aldehydes* **8**, **51**, *and* **52**, *followed by* ^{1}*H-NMR spectroscopy.* Each compound 15 mM, pD 5.0, 20°.

Aldehyde hydrates **8**, **51**, and **52** were formed *in situ* by monitoring the deprotection of stock solutions of the corresponding acetals using DCl. Upon dilution with acetate buffer (pD 5.0), **8** and **52** reacted with hydrazide **7** to give only 50% acylhydrazone formation at equilibrium and with a $t_{1/2}$ value of 20 min (*Fig. 3*). In contrast, aldehyde hydrate **51** reacted with **7** to give over 70% product formation at equilibrium with a $t_{1/2}$ value of *ca.* 2 min. Other α-amido aldehydes similar to **51** have been found to react readily with the hydrazide functionality [19b]. This difference in reactivity may be due to steric hindrance of the aldehyde functionalities in aldehyde hydrates **8** and **52** compared with **51** or to the α-amido NH bond which may stabilise the intermediate hemi-aminal. Whatever the determining factor, the data show that subtle modifications in aldehyde substituents can considerably alter their ability to condense with hydrazides in aqueous media. Furthermore, the degree of polymerisation (DP) in closed-system equilibrium polymerisation reactions is related to the degree of product formation such that 50% acylhydrazone formation can only be expected to give a DP = 2 [32]. Thus, high-yielding reactions or higher concentrations are necessary in order to access long oligomers or polymers. Experiments are now underway in our laboratory to evaluate whether these short oligomers can polymerise in the presence of complementary ssDNA. The ability of the other bifunctionalised components of type **II**–**V** to oligomerise in aqueous media is also being investigated.

2.3. *Synthesis of Monofunctionalised Components.* To decorate a polymer or core group functionalised with either aldehyde or hydrazide side-chain groups, nucleoside or nucleobase derivatives containing either a hydrazide or aldehyde group respectively, **VI**–**X** were synthesised (*Fig. 4*).

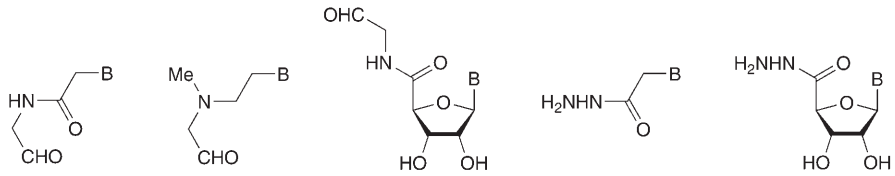
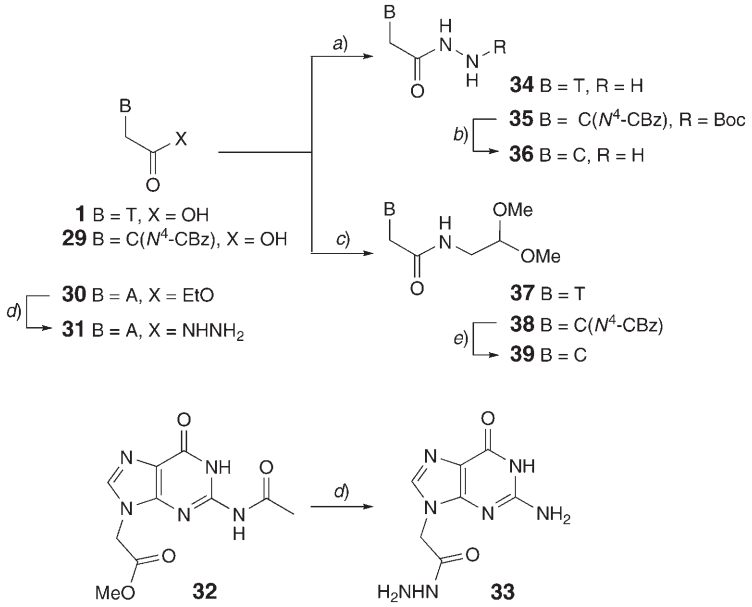


Fig. 4. *Overview of monofunctional nucleobase/nucleoside derivatives* **VI**–**X**

Since NH_2NH_2 readily undergoes an addition reaction with the pyrimidine bases, the hydrazide-functionalised nucleobases **34** and **36** were obtained by first coupling the nucleobase acids **1** and **29** [24a], respectively, with NH_2NHCOO^tBu using *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC) (*Scheme 6*). The thymidyl intermediate was then treated directly with aq. HCl to give the hydrazide **34**, while the CBz protecting group on cytidyl intermediate **35** was first removed by hydrogenation using cyclohexene as a hydrogen source over Pd/C, and then likewise treated with 1M aq. HCl to give the hydrazide **36**. Purine bases are, however, much less susceptible to nucleophilic attack, and thus ester **30** [33] was converted directly into hydrazide **31** upon simple treatment with excess $NH_2NH_2 \cdot H_2O$ (*Scheme 6*). Likewise, guanine hydrazide **33** was formed by hydrazinolysis of the ester **32** [34]. Acetals **37** and

Scheme 6. *Synthesis of the Hydrazido- and Acetal-Functionalised Derivatives* **31**, **33**, **34**, **36**, **37**, *and* **39**

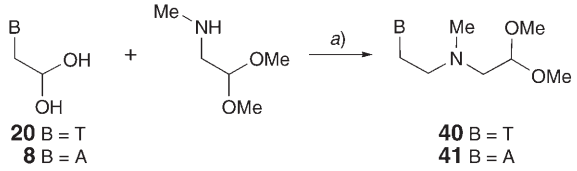


a) NH_2NHCOO^tBu, *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC), DMF, 20°; then for **1**, 1M aq. HCl, 20°. *b*) 10% Pd/C, cyclohexene, EtOH, reflux; then 1M aq. HCl, 20°. *c*) Aminoacetaldehyde dimethyl acetal, EDC, DMF, 20°. *d*) $NH_2NH_2 \cdot H_2O$, MeOH, reflux. *e*) 10% Pd/C, cyclohexene, EtOH, reflux.

38 were obtained *via* EDC coupling of the acids **1** and **29** with aminoacetaldehyde dimethyl acetal, respectively. Hydrogenation of CBz-protected **38** gave the acetal **39** in near quantitative yield.

Reductive amination of thymine aldehyde hydrate **20** or adenine aldehyde hydrate **8** with (methylamino)acetaldehyde dimethyl acetal gave the corresponding acetals **40** and **41**, respectively (*Scheme 7*).

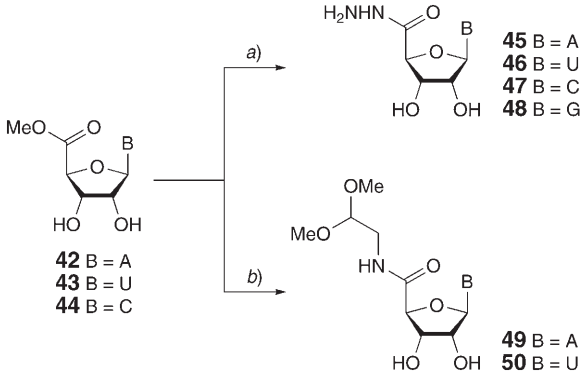
Scheme 7. *Synthesis of the Acetal-Functionalised Nucleobase Derivatives* **40** *and* **41**



a) $NaCNBH_3$, AcOH, r.t

The 5′-(hydrazinocarbonyl)adenosine **45** (*Scheme 8*) was synthesised as reported in [35]. The 5′-(hydrazinocarbonyl)nucleoside analogues **46** and **47** were obtained from the esters **43** and **44**, respectively [36][37], by hydrazinolysis in MeOH or EtOH. Synthesis of the corresponding guanosine derivative **48** is described in [12c]. The acetals **49** and **50** were obtained by heating esters **42** and **43**, respectively, in aminoacetaldehyde dimethyl acetal as a solvent. Acetals **49** and **50** are also intriguing for their potential to form polyacetals in the presence of complementary *ss*DNA *via* self-condensation of the aldehyde with the 2′,3′-diol functionality of the ribose ring.

Scheme 8. *Synthesis of Hydrazido- and Acetal-Functionalised Ribonucleosides* **45**–**50**



a) $NH_2NH_2 \cdot H_2O$, MeOH, 20°. *b*) Aminoacetaldehyde dimethyl acetal, 60–70°.

Acylhydrazone Formation. To investigate the suitability of such monofunctionalised components for DCC, nucleobase aldehyde hydrate **54** was condensed with hydrazides **34**, **36**, and **57** to give the corresponding acylhydrazones in 76, 72, and 60% yield, respectively, at equilibrium and with a $t_{1/2}$ value of maximum 7 min (*Fig. 5* and *Table*). Nucleobase aldehyde hydrates **53**–**55** were next condensed with valine hydrazide **56** [19b] at pD 5.0 and 15 mM concentration each, and the reaction was followed by ^{1}H-NMR spectroscopy. Aldehyde hydrates **53**–**55** all formed acylhydrazones very fast

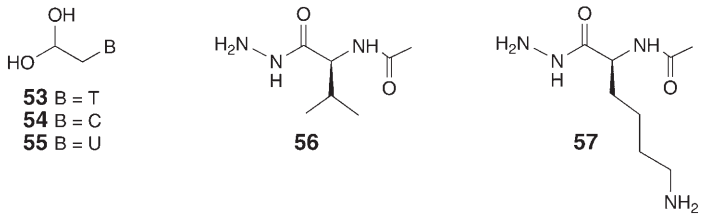


Fig. 5. *Aldehyde- and hydrazide-functionalised nucleobase and amino acid derivatives*

Table. *Acylhydrazone Formation of Selected Nucleobase Aldehyde and Hydrazide Derivatives, and Then Exchange upon Addition of a Second Hydrazide* **57** *Determined by* ^{1}*H-NMR Spectroscopy*[a])

Aldehyde	Hydrazide	Yield of acylhydrazone [%]	$t_{1/2}$(formation) [min]	$t_{1/2}$(exchange) [min]
53	**56**	65	12	20–25
54	**34**	76	5	n.d.[b])
54	**36**	72	<2	n.d.
54	**57**	60	7	n.d.
54	**56**	62	<2	10
55	**56**	67	6	20–25

[a]) Concentration of each compound 15 mM, pD 5.0, 20°. [b]) n.d.: Not determined.

($t_{1/2} < 15$ min) and gave equilibrium yields of 62–67% (*Table*), demonstrating that such compounds are sufficiently isoenergetic for use in DCC libraries. Lysine hydrazide **57** [19b] was then added to the solution, and the exchange was again monitored by ^{1}H-NMR spectroscopy. As can be seen from the *Table*, acylhydrazones formed from aldehyde hydrates **53**–**55** all underwent efficient hydrazide exchange upon addition of lysine hydrazide **57** at pD 5.0.

3. Conclusions. – A variety of mono- and bifunctionalised nucleic acid components have been synthesised for application in dynamic combinatorial chemistry (DCC). Preliminary data have been obtained on the ability of these components to generate dynamic nucleic acids (DyNAs) [6b], reversible polymers bearing nucleobase groups. Further exploration of these features is warranted in order to evaluate the potential of such dynamic analogues of biopolymers.

This work was supported by a *Marie-Curie Human Potential Individual Fellowship* (*D. T. H.*), the *Collège de France* (*D. T. H.* and *N. S.*), and the *French Ministry of Research* (*N. S.*).

Experimental Part

General. All reagents were purchased from commercial suppliers and used without any further purification. TLC: *Polygram Sil-G/UV$_{254}$* pre-coated plastic sheets. Flash chromatography (FC): 230–400-mesh silica-gel particles from *Merck*. M.p.: *Büchi B-540* melting-point apparatus; uncorrected. ^{1}H- (200 or 400 MHz) and ^{13}C-NMR (50 or 101 MHz) spectra: *Bruker AC-200*, *Avance-400* spectrometers, resp.; chemical shifts δ in ppm downfield from TMS ($\delta = 0$); coupling constants J in Hz. Electrospray-ionisation mass spectrometry (ESI-MS): *Bruker Micro-TOF* mass spectrometer; fast-atom-bombardment (FAB) MS, and low- or high-resolution (HR) MS: carried out by the Service d'Analyse de

l'Université Louis Pasteur, in *m/z* (rel.%). Microanalyses were performed by the Service Central de Microanalyse du CNRS, Faculté de Chimie, Strasbourg.

*Methyl 2-{(2,2-Dimethoxyethyl)[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetyl]amino}acetate* (**4**). To a soln. of *methyl 2-[(2,2-dimethoxyethyl)amino]acetate* (**3**) in DMF (10.0 ml) was added HOBt (1.21 g, 8.93 mmol), followed by *N*-methylmorpholine (980 µl, 17.9 mmol). To this soln. was then added a soln. of *(thymin-1-yl)acetic acid* (**1**; 1.64 g, 8.93 mmol) in DMF (10 ml) and then diisopropylcarbodiimide (1.67 ml, 10.7 mmol). The soln. was stirred at r.t. for 16 h, then the resulting cloudy soln. was filtered. To the filtrate, H_2O (5 ml) was added, and the soln. was evaporated to dryness and then extracted from H_2O (50 ml) with $CHCl_3$/EtOH 2:1 (total 5 × 50 ml). The org. fractions were combined, washed with brine (50 ml), and dried (Na_2SO_4). After filtration and evaporation, the crude product was purified by FC (SiO_2; AcOEt) to give **4** (2.58 g, 84%). White solid. M.p. 103–105°. ^{1}H-NMR (200 MHz, $CDCl_3$): 9.34, 9.29 (2*s*, total 1 H); 7.03, 6.98 (2*s*, total 1 H); 4.68, 4.47 (2*s*, total 2 H); 4.52, 4.33 (2*t*, J = 5.1, 4.9, total 1 H); 4.24, 4.15 (2*s*, total 2 H); 3.78, 3.70 (2*s*, total 3 H); 3.53–3.46 (*m*, 2 H); 3.45, 3.34 (2*s*, total 6 H); 1.90, 1.89 (2*d*, J = 1.1, total 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.0; 169.6; 168.0; 164.8; 151.5; 141.5; 141.3; 103.4; 103.3; 110.3; 110.6; 55.6; 54.9; 52.6; 52.3; 51.2; 50.6; 50.4; 49.4; 48.1; 47.9; 12.3. FAB-MS: 344.1 ([M + H]$^+$, 65), 312.1 (100). HR-FAB-MS: 344.1462 ($C_{14}H_{22}N_3O_7^+$; calc. 344.1458).

Methyl 2-{[2-(6-(Benzoylamino)purin-9-yl)acetyl](2,2-dimethoxyethyl)amino}acetate (**5**). Procedure as for **4** gave **5** (630 mg, 57%). White solid. M.p. 80–81°. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.08 (*s*, 1 H); 8.84, 8.83 (2*s*, total 1 H); 8.21, 8.17 (2*s*, total 1 H); 8.07, 8.05 (2*s*, total 2 H); 7.68–7.50 (*m*, total 3 H); 5.36, 5.12 (2*s*, total 2 H); 4.61, 4.39 (2*t*, J = 5.0, 4.9, total 1 H); 4.40, 4.23 (2*s*, total 2 H); 3.88, 3.77 (2*s*, total 3 H); 3.67, 3.57 (2*d*, J = 5.3, 4.7, total 2 H); 3.56, 3.39 (2*s*, total 6 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 169.6; 169.2; 167.0; 166.9; 164.7; 152.6; 152.2; 149.4; 144.2; 144.1; 133.8; 133.7; 132.7; 132.6; 128.8; 127.9; 122.2; 103.4; 103.3; 55.7; 55.1; 52.8; 52.3; 51.5; 50.8; 50.4; 49.5; 44.0; 43.8; 42.1; 23.5. FAB-MS: 457.2 ([M + H]$^+$, 100). HR-FAB-MS: 457.1834 ($C_{21}H_{25}N_6O_6^+$; calc. 457.1836).

N-*(2,2-Dimethoxyethyl)*-N-*[(hydrazinocarbonyl)methyl]-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamide* (**6**). To a soln. of **4** (207 mg, 0.60 mmol) in EtOH (3 ml) was added $NH_2NH_2 \cdot H_2O$ (146 µl, 3.02 mmol), and the soln. was stirred at r.t. for 8 h. The resulting precipitate was filtered and washed extensively with EtOH. Drying under vacuum gave **6** (130 mg, 63%). White solid. M.p. 124–126°. ^{1}H-NMR (200 MHz, D_2O): 7.40, 7.37 (2*s*, total 1 H); 4.88, 4.73 (2*s*, total 2 H); 4.70, 4.54 (2*t*, J = 4.7, 3.7, total 1 H); 4.27, 4.14 (2*s*, total 2 H); 3.63 (*d*, J = 4.4, 1 H); 3.53 (*s*, 5 H); 3.43 (*s*, 2 H); 1.90 (*s*, 3 H). ^{13}C-NMR (50 MHz, D_2O): 170.7; 170.6; 170.2; 169.5; 167.6; 152.9; 152.9; 143.9; 143.8; 111.6; 111.5; 104.2; 103.6; 56.8; 55.8; 51.6; 50.7; 50.6; 50.5; 50.1; 49.9; 12.0. ESI-MS: 687.3109 ([2M + H]$^+$, $C_{13}H_{21}N_5O_6$; calc. 343.1486). Anal. calc. for $C_{13}H_{21}N_5O_6$: C 45.48, H 6.17, N 20.40; found: $C_{13}H_{21}N_5O_6 \cdot 0.1\ H_2O$: C 45.24, H 6.19, N 20.29.

2-(6-Aminopurin-9-yl)-N-*(2,2-dimethoxyethyl)*-N-*[(hydrazinocarbonyl)methyl]acetamide* (**7**). To a soln. of **5** (700 mg, 1.54 mmol) in EtOH (20 ml) was added $NH_2NH_2 \cdot H_2O$ (373 µl, 7.68 mmol), and the soln. was stirred at r.t. for 48 h. The resulting precipitate was filtered and washed with cold EtOH (10 ml) to give **7** (363 mg, 67%). White solid. M.p. 190–192°. ^{1}H-NMR (400 MHz, D_2O): 8.09 (*s*, 1 H); 7.98, 7.97 (2*s*, total 1 H); 5.28, 5.15 (2*s*, total 2 H); 4.66, 4.42 (2*t*, J = 4.9, 4.5, total 1 H); 4.27, 4.05 (2*s*, total 2 H); 3.64, 3.44 (2*d*, J = 5.3, 4.7, total 2 H); 3.46, 3.31 (2*s*, total 6 H). ^{13}C-NMR (101 MHz, D_2O): 170.1; 170.1; 170.0; 169.4; 155.8; 153.1; 149.6; 149.6; 143.4; 118.4; 104.1; 103.5; 56.7; 55.7; 51.6; 50.8; 50.6; 45.4. FAB-MS: 353.1 ([M + H]$^+$, 100), 321.1 (20). HR-FAB-MS: 353.1685 ([M + H]$^+$, $C_{13}H_{21}N_8O_4^+$; calc. 353.1686).

Methyl 2-{[2-(6-Aminopurin-9-yl)ethyl](2,2-dimethoxyethyl)amino}acetate (**9**). To a suspension of *(adenin-9-yl)ethanal hydrate hydrochloride* (**8**; 125 mg, 0.54 mmol) in MeOH (5 ml) was added **3** (96 mg, 0.54 mmol) in MeOH (2 ml), followed by $NaCNBH_3$ (68 mg, 1.08 mmol) and then AcOH (123 µl, 2.16 mmol). The mixture was stirred at r.t. for 6 h, and then the reaction was quenched with sat. aq. $NaHCO_3$ (5 ml). AcOEt (30 ml) and H_2O (30 ml) were added. The org. phase was extracted (total 3 × 30 ml of AcOEt), and the org. fractions were combined, washed with sat. brine, and dried (Na_2SO_4). After filtration and evaporation, the crude product was purified by FC (SiO_2; 5 → 10% MeOH in CH_2Cl_2) to give **9** (89 mg, 49%). White solid. M.p. 96–98°. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.32 (*s*, 1 H); 8.03 (*s*, 1 H); 6.00 (*s*, 2 H); 4.20–4.26 (*m*, 3 H); 3.63 (*s*, 3 H); 3.40 (*s*, 2 H); 3.27 (*s*, 6 H); 3.13 (*t*, J = 5.7, 2 H); 2.80 (*d*, J = 5.0, 2 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 171.9; 155.9; 152.9; 150.1; 141.9; 119.5; 104.2; 56.6; 56.2; 55.3; 54.1; 51.6; 42.6. FAB-MS: 339.1 ([M + H]$^+$, 100). HR-FAB-MS: 339.1774 ([M + H]$^+$,

$C_{14}H_{23}N_6O_4^+$; calc. 339.1781). Anal. calc. for $C_{14}H_{22}N_6O_4$: C 49.70, H 6.55, N 24.84; found: C 49.57, H 6.71, N 24.44.

2-{[2-(6-Aminopurin-9-yl)ethyl](2,2-dimethoxyethyl)amino}acetohydrazide (**10**). To a soln. of **9** (69 mg, 0.204 mmol) in EtOH (4 ml) was added $NH_2NH_2 \cdot H_2O$ (250 µl, 5.10 mmol), and the soln. was stirred at r.t. for 24 h and then evaporated under reduced pressure to give **10** (68 mg, 99%). White solid. M.p. 170–172°. ^{1}H-NMR (400 MHz, D_2O): 8.24 (*s*, 1 H); 8.14 (*s*, 1 H); 4.30 (*dd*, *J* = 5.6, 5.6, 2 H); 3.93 (*t*, *J* = 5.3, 1 H); 3.33 (*s*, 2 H); 3.13 (*s*, 6 H); 3.07 (*dd*, *J* = 5.6, 5.6, 2 H); 2.56 (*d*, *J* = 5.3, 2 H). ^{13}C-NMR (101 MHz, CD_3OD): 172.7; 157.4; 153.7; 150.9; 143.4; 120.0; 105.1; 58.7; 58.6; 56.6; 54.8; 43.6. FAB-MS: 339.1 ([*M* + H]$^+$, 100). HR-FAB-MS: 339.1895 ([*M* + H]$^+$, $C_{13}H_{23}N_8O_3^+$; calc. 339.1893). Anal. calc. for $C_{13}H_{22}O_3N_8 \cdot 0.5\ H_2O$: C 44.95, H 6.67, N 32.26; found: C 45.00, H 6.81, N 32.12.

*(9*H*-Fluoren-9-yl)methyl 2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-6-(hydroxymethyl)morpholine-4-carboxylate* (**13**). To a suspension of *2-(2,4-dioxo-3,4-dihydropyridmidin-1(2*H*)-yl)-6-(hydroxymethyl)morpholine* (**12**; 0.82 g, 3.61 mmol) in THF (20 ml) was added $(i\text{-}Pr)_2NH$ (753 µl, 4.33 mmol), and the soln. was cooled in an ice-bath. Fmoc chloride (0.98 g, 3.79 mmol) was then added, and, after a couple of min, the temp. was raised to r.t. Stirring was continued for a further 2 h, and then to the resulting homogenous soln. was added H_2O (2 ml), and the org. solvents were then removed under reduced pressure. AcOEt (30 ml) and H_2O (30 ml) were then added, and the org. phase was isolated. After repeated extraction with AcOEt (5 × 30 ml), the org. phases were combined and dried (Na_2SO_4). Filtration and evaporation gave a crude product, which was purified by FC (SiO_2; AcOEt) to give **13** (1.50 g, 93%). White solid. M.p. > 140° (dec.). ^{1}H-NMR (200 MHz, CD_3OD): 7.84–7.77 (*m*, 2 H); 7.72 (*d*, *J* = 4.0, 1 H); 7.64–7.57 (*m*, 2 H); 7.45–7.30 (*m*, 4 H); 5.71 (*d*, *J* = 3.6, 1 H); 5.68–5.47 (br. *m*, 1 H); 4.49 (*dd*, *J* = 3.0, 5.2, 1 H); 4.42–4.30, 4.75–4.60 (2 br. *m*, total 1 H); 4.24 (*t*, *J* = 3.0, 1 H); 4.17–4.00 (*m*, 1 H); 3.80–3.40 (*m*, 4 H); 2.95–2.70 (*m*, 2 H). ^{13}C-NMR (101 MHz, CD_3OD): 164.5; 155.1; 150.1; 143.7; 141.2; 140.6; 127.4; 126.9; 124.7; 119.6; 101.5; 79.1; 76.9; 61.7; 53.4. FAB-MS: 450 ([*M* + H]$^+$, 100), 179.2 (100). HR-FAB-MS: 450.1666 ([*M* + H]$^+$, $C_{24}H_{24}N_3O_6^+$; calc. 450.1665).

*(9*H*-Fluoren-9-yl)methyl 2-(Dimethoxymethyl)-6-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholine-4-carboxylate* (**14**). To a soln. of **13** (0.78 g, 1.74 mmol) in dry CH_2Cl_2 (30 ml) was added the *Dess–Martin* periodinane reagent (0.96 g, 2.26 mmol) in two portions separated by 15 min. The soln. was then stirred under Ar for 12 h, and then a further quantity of the *Dess–Martin* periodinane (0.52 g, 1.22 mmol) was added, again in two portions separated by 15 min. Stirring was continued for a further 2 h, whereupon the suspension was filtered, and to the filtrate was added MeOH (2 ml), and then the soln. was evaporated under reduced pressure. Purification by FC (SiO_2; MeOH/CH_2Cl_2 1:19) gave the corresponding aldehyde (0.55 g, 71%) as a white solid. To a soln. of this aldehyde (465 mg, 1.04 mmol) in MeOH (5 ml) were added trimethyl orthoformate (5 ml) and then TsOH (198 mg, 1.04 mmol). The soln. was stirred at r.t. for 30 min and then cooled in an ice-bath. The reaction was quenched by the dropwise addition of an aq. soln. of Na_2CO_3 (10% (*w*/*v*), 20 ml), followed by AcOEt (30 ml). The org. phase was separated and extracted from the aq. phase (total 4 × 30 ml of AcOEt). The org. fractions were combined, washed with brine, dried (Na_2SO_4), filtered and evaporated under vacuum. Purification by FC (SiO_2; AcOEt/hexane 3:2) gave **14** (461 mg, 90%). White solid. M.p. 112–114°. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.86 (br., 1 H); 7.20–7.80 (*m*, 9 H); 5.77 (*d*, 1 H); 5.67 (*dd*, *J* = 10.0, 2.9, 1 H); 4.60–4.20 (*m*, 6 H); 3.74–3.62 (*m*, 1 H); 3.43 (*d*, *J* = 3.3, 6 H); 2.95–2.65 (*m*, 2 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 163.3; 154.8; 149.8; 143.7; 141.2; 139.2; 127.7; 127.1; 125.0; 120.0; 103.1; 102.9; 55.5; 54.1; 47.0. FAB-MS: 494.3 ([*M* + H]$^+$, 17), 179.2 (100); HR-FAB-MS: 494.1925 ([*M* + H]$^+$, $C_{26}H_{28}N_3O_7^+$; calc. 494.1927).

*1-[6-(Dimethoxymethyl)morpholin-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione* (**15**). A soln. of **14** (340 mg, 0.69 mmol) in a 5% (*v*/*v*) soln. of piperidine in THF (total volume 5 ml) was stirred at r.t. for 8 h, and then evaporated under reduced pressure. Purification by FC (SiO_2; MeOH/CH_2Cl_2 1:9) gave **15** (178 mg, 95%). White solid. M.p. 193–195°. ^{1}H-NMR (400 MHz, CD_3OD): 7.73 (*d*, *J* = 8.2, 1 H); 5.73 (*d*, *J* = 8.2, 1 H); 5.72 (*dd*, *J* = 2.6, 2.6, 1 H); 4.36 (*d*, *J* = 5.8, 1 H); 3.83–3.91 (*m*, 1 H); 3.42, 3.44 (2*s*, total 6 H); 3.01 (*dd*, *J* = 12.6, 2.6, 1 H); 2.93 (*dd*, *J* = 13.2, 2.6, 1 H); 2.72 (*dd*, *J* = 10.2, 10.2, 1 H); 2.63 (*dd*, *J* = 10.8, 10.8, 1 H). ^{13}C-NMR (101 MHz, CD_3OD): 164.5; 150.3; 140.8; 104.0; 101.4; 80.1; 77.4; 54.5; 53.2; 44.0. FAB-MS: 272.2 ([*M* + H]$^+$, 100). HR-FAB-MS: 272.1245 ([*M* + H]$^+$, $C_{11}H_{18}N_3O_5^+$; calc. 272.1246).

tert-*Butyl* N′*-[2-(Dimethoxymethyl)-6-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)morpholine-4-carbonyl]hydrazinecarboxylate* (**16**). To a soln. of *N*,*N*′-carbonyldiimidazole (23 mg, 0.14 mmol) in dry DMF

(3 ml) was added NH_2NHCOO^tBu (19 mg, 0.14 mmol), and the soln. was stirred at r.t. under Ar for 5 h. A soln. of **15** (35 mg, 0.13 mmol) in dry DMF (3 ml) was then added, and the soln. was stirred at r.t. for a further 1 h. The reaction was quenched by the addition of a 10% (*w*/*v*) aq. soln. of $NaHCO_3$ (20 ml). AcOEt (20 ml) was then added, and the org. phase was extracted (total 5×20 ml of AcOEt). The org. fractions were combined, washed with brine, and dried (Na_2SO_4). Filtration and evaporation gave a crude product, which was purified by FC (SiO_2; MeOH/CH_2Cl_2 1:19) to give **16** (44 mg, 80%). White solid. M.p. $>118°$ (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.65 (br. *s*, 1 H); 7.43 (*d*, $J = 8.2$, 1 H); 6.85 (*s*, 1 H); 6.41 (*d*, $J = 3.2$, 1 H); 5.76 (*d*, $J = 8.2$, 1 H); 5.72 (*dd*, $J = 2.9, 2.9$, 1 H); 4.35 (*d*, $J = 5.2$, 1 H); 4.20–4.12 (*m*, 1 H); 4.00–3.85 (*m*, 2 H); 3.45, 3.41 (2*s*, total 6 H); 2.93–2.78 (*m*, 2 H); 1.47 (*s*, 9 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 163.5; 157.8; 156.9; 150.2; 139.6; 103.6; 103.2; 81.6; 79.4; 75.7; 55.8; 54.6; 46.9; 43.4; 28.3. FAB-MS: 452.3 ($[M + Na]^+$, 10), 430.3 ($[M + H]^+$, 72), 342.2 (100). HR-FAB-MS: 430.1931 ($[M + H]^+$, $C_{17}H_{28}N_5O_8^+$; calc. 430.1938).

*Benzyl 4-{*N′*-[(*tert*-Butoxy)carbonyl]hydrazino}-3-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-4-oxobutanoate* (**18**). To a soln. of Fmoc-L-Asp(OBzl)-OH (**17**; 3 g, 6.74 mmol) in dry CH_2Cl_2 (30 ml) were added HOBt (0.91 g, 6.74 mmol), NH_2NHCOO^tBu (0.89 g, 6.74 mmol), and then diisopropylcarbodiimide (1.05 ml, 6.74 mmol). The soln. was stirred at r.t. under Ar for 14 h, during which time a fine precipitate formed. This was filtered through a plug of *Celite*, and to the filtrate was added H_2O (30 ml). The org. phase was extracted (total 3×50 ml of CH_2Cl_2), the org. fractions were combined, washed with brine, and dried (Na_2SO_4). Filtration and evaporation under reduced pressure gave a crude product that was recrystallised (hexane/AcOEt) to give **18** (3.00 g, 80%). White crystalline solid. M.p. 151–152°. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.29 (br. *s*, 1 H); 7.78 (*d*, $J = 7.6$, 2 H); 7.59 (*d*, $J = 7.3$, 2 H); 7.30–7.50 (*m*, 9 H); 6.48 (br. *s*, 1 H); 6.03 (br. *m*, 1 H); 5.17 (*s*, 2 H); 4.72 (*m*, 1 H); 4.45 (*d*, $J = 5.6$, 2 H); 4.22 (*t*, $J = 6.5$, 1 H); 3.04 (*m*, 1 H); 2.84 (*m*, 1 H); 1.48 (*s*, 9 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 171.3; 170.4; 156.3; 155.2; 143.7; 141.3; 135.4; 128.6; 128.4; 128.3; 127.8; 127.2; 125.2; 120.0; 81.8; 67.4; 67.0; 50.0; 47.1; 36.3; 28.2. FAB-MS: 582.1 ($[M + Na]^+$, 25), 560.2 ($[M + H]^+$, 25). HR-FAB-MS: 560.2391 ($[M + H]^+$, $C_{31}H_{34}N_3O_7^+$; calc. 560.2397). Anal. calc. for $C_{31}H_{33}N_3O_7$: C 66.53, H 5.94, N 7.51; found: C 66.47, H 6.00, N 7.18.

tert-*Butyl* N′*-{2-Amino-4-[(2,2-dimethoxyethyl)amino]-4-oxobutanoyl}hydrazinecarboxylate* (**19**). To a soln. of **18** (0.95 g, 1.70 mmol) in THF (10 ml) was added aminoacetaldehyde dimethyl acetal (364 µl, 3.40 mmol), and the soln. was stirred at r.t. for 48 h. The soln. was evaporated under reduced pressure, and MeOH was added (10 ml), and the resulting fine white suspension was removed by filtration. The filtrate was evaporated under reduced pressure and purified by FC (SiO_2; 10 → 15% MeOH in CH_2Cl_2) to give **19** (400 mg, 70%). Waxy solid. ^{1}H-NMR (400 MHz, CD_3OD): 4.46 (*t*, $J = 5.5$, 1 H); 3.75 (*dd*, $J = 4.7, 4.7$, 1 H); 3.41 (*s*, 6 H); 3.38–3.31 (*m*, 2 H); 2.47 (*dd*, $J = 4.7, 4.7$, 1 H); 2.46 (*dd*, $J = 8.5, 8.5$, 1 H); 1.49 (*s*, 9 H). ^{13}C-NMR (101 MHz, CD_3OD): 176.1; 172.9; 157.7; 103.7; 81.9; 54.5; 54.5; 53.2; 42.0; 40.0; 28.5. FAB-MS: 335.1 ($[M + H]^+$, 100). HR-FAB-MS: 335.1922 ($[M + H]^+$, $C_{13}H_{27}N_4O_6^+$; calc. 335.1931).

tert-*Butyl* N′*-(4-[(2,2-Dimethoxyethyl)amino]-2-{[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)ethyl]amino}-4-oxobutanoyl)hydrazinecarboxylate* (**21**). (Thymin-1-yl)ethanal hydrate (**20**; 33 mg, 0.21 mmol) was added to a soln. of **19** (70 mg, 0.21 mmol) in MeOH (1 ml), and, after 12 h of stirring at r.t., $NaCNBH_3$ (26 mg, 0.42 mmol) and AcOH (13 µl, 0.21 mmol) were added. Stirring was continued for 48 h, and then the soln. was evaporated under reduced pressure and purified by FC (SiO_2; 5 → 10% MeOH in CH_2Cl_2) to give **21** (30 mg, 31%). White solid. M.p. 76–77°. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.00 (br. *s*, 1 H); 7.60 (br. *s*, 1 H); 7.20 (*s*, 1 H); 7.20 (*s*, 1 H); 4.44 (*t*, $J = 5.0$, 1 H); 3.90 (*m*, 2 H); 3.74 (*m*, 1 H); 3.50–3.30 (*m*, 8 H); 3.03 (*m*, 2 H); 2.75 (*m*, 2 H); 1.92 (*s*, 3 H); 1.47 (*s*, 9 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 170.6; 164.4; 155.6; 151.5; 141.0; 110.8; 102.1; 81.7; 58.8; 54.0; 53.9; 47.9; 46.2; 40.7; 36.0; 28.2; 12.2. HR-ESI-MS (pos.): 487.2510 ($[M + H]^+$, $C_{20}H_{35}N_6O_8^+$; calc. 487.2511).

2-(Acetoxymethyl)-5-{[(benzyloxy)carbonyl]amino}tetrahydropyran-3,4,6-triyl Triacetate (**23**). To a soln. of **22** (16.5 g, 53 mmol) in pyridine (290 ml) at 0° was added Ac_2O (40 ml), and the soln. was stirred for 30 min, then the temp. was raised to r.t., and the soln. was stirred for a further 16 h. H_2O (1 ml) was then added, and, after 10 min, the soln. was evaporated under reduced pressure. AcOEt (100 ml) was then added, followed by H_2O (100 ml), and the org. phase was separated. After repeated extraction with AcOEt (total 4×100 ml), the org. fractions were pooled, washed with brine (100 ml), and dried

($MgSO_4$). Filtration and evaporation gave a residue which was filtered through a plug of silica to remove excess pyridine. To the resulting oil was added Et_2O (300 ml). The soln. was left to crystallise at r.t. for 30 min and then for 16 h at 5°. The resulting crystals were filtered and washed with Et_2O (50 ml) and dried under reduced pressure to give **23** (5.61 g, 22%). White crystalline solid. M.p. 151–152°. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.33 (br. *s*, 5 H); 5.67 (*d*, *J* = 8.4, 1 H); 5.09 (*s*, 2 H); 4.94–4.79 (*m*, 1 H); 4.35–3.70 (*m*, 5 H); 2.08 (*s*, 3 H); 2.03–2.00 (br. *s*, 6 H); 1.95 (*s*, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.8; 170.6; 169.4; 155.9; 136.4; 128.4; 128.1; 127.9; 92.4; 72.5; 68.2; 66.7; 61.7; 54.7; 20.6; 20.5. FAB-MS: 422.4 ([*M* + H – (4 CH_3)]$^+$, 100). Anal. calc. for $C_{22}H_{27}NO_{11}$: C 54.88, H 5.65, N 2.91; found: C 55.04, H 5.46, N 2.91.

*2-(Acetoxymethyl)-5-{[(benzyloxy)carbonyl]amino}-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydropyran-3,4-diyl Diacetate* (**24**). To a suspension of thymine (1.83 g, 14.6 mmol) in MeCN (10 ml) was added *N*,*O*-bis[(trimethylsilyl)acetamide] (7.54 ml, 30.6 mmol)), and the soln. was stirred at r.t. for 10 min during which time a homogenous soln. was formed. Compound **23** (3.50 g, 7.28 mmol) was then added, followed by trimethylsilyl trifluoromethanesulfonate (2.9 ml, 16.0 mmol), and the soln. was heated to 80° for 2 h under Ar. The flask was then cooled to r.t., and a 5% (*w*/*v*) aq. soln. of $NaHCO_3$ (100 ml) was added, followed by AcOEt (100 ml). The org. phase was extracted with AcOEt (total 4 × 100 ml), and these fractions were combined, washed with brine, and dried (Na_2SO_4). Filtration and evaporation gave a crude product which was purified by FC (40% hexane in AcOEt) to give **24** (3.29 g, 83%). White solid. M.p. 110–112°. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.27 (*s*, 5 H); 7.19 (*s*, 1 H); 5.84, 5.81 (2*d*, *J* = 9.9, 10.2, total 2 H); 5.37 (*t*, *J* = 9.9, 1 H); 5.12 (*t*, *J* = 9.9, 1 H); 4.98 (*d*, *J* = 2.6, 2 H); 4.24 (*dd*, *J* = 12.4, 4.7, 1 H); 4.16–4.00 (*m*, 2 H); 3.90–3.77 (*m*, 1 H); 2.08, 2.01, 1.93, 1.90 (4*s*, each 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.7; 169.7; 164.7; 163.7; 156.5; 151.6; 151.5; 140.0; 136.4; 135.8; 129.0; 128.5; 128.3; 128.2; 128.0; 127.7; 112.1; 111.2; 81.2; 74.6; 73.0; 68.4; 67.0; 62.1; 53.9; 50.9; 50.2; 20.7; 20.5; 20.4; 12.5; 12.3. FAB-MS: 548.4 ([*M* + H]$^+$, 100), 217.3 (60). Anal. calc. for $C_{25}H_{29}N_3O_{11}$: C 54.84, H 5.34, N 7.67; found: C 54.79, H 5.14, N 7.64.

*Benzyl [4,5-Dihydroxy-6-(hydroxymethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydropyran-3-yl]carbamate* (**25**). To **24** (20 mg, 0.04 mmol) was added Et_3N/H_2O/MeOH 1 : 4 : 5 (total 2 ml), and the soln. was stirred at r.t. for 2 h. The soln. was evaporated under reduced pressure and purified by FC (10% MeOH/CH_2Cl_2) to give **25** (14 mg, 90%). White solid. M.p. 84–85°. ^{1}H-NMR (200 MHz, CD_3OD): 7.55 (*s*, 1 H); 7.35–7.15 (*m*, 5 H); 5.63 (*d*, *J* = 9.5, 1 H); 5.02–4.90 (*m*, 2 H); 3.92–3.30 (*m*, 6 H); 1.84 (*s*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 164.1; 156.5; 151.3; 137.7; 137.6; 128.7; 128.0; 127.4; 109.3; 81.6; 80.3; 74.2; 70.5; 65.4; 61.4; 55.5; 46.2; 23.0; 12.6. HR-ESI-MS (pos.) 444.1401 ([*M* + Na]$^+$, $C_{19}H_{23}N_3NaO_8^+$; calc. 444.1377).

*Benzyl [5-(Hydroxymethyl)-2,3-dimethoxy-2,3-dimethyl-7-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)hexahydro-5*H*-pyrano[3,4-*b*][1,4]dioxin-8-yl]carbamate* (**26**). To a soln. of **25** (1.20 g, 2.85 mmol) in MeOH (50 ml) were added butane-2,3-dione (275 µl, 3.14 mmol), (+)-camphor-10-sulfonic acid monohydrate (143 mg, 0.57 mmol), and trimethyl orthoformate (934 µl, 8.55 mmol), and the mixture was heated at reflux for 16 h. The reaction was quenched by the addition of Et_3N (1.0 ml), and the soln. was evaporated to dryness under reduced pressure. Purification by FC (5 → 15% MeOH/CH_2Cl_2) gave **26** (1.00 g, 72%). White solid. M.p. 158–159°. ^{1}H-NMR (200 MHz, $CDCl_3$): 10.61, 10.46 (2*s*, total 1 H); 7.35–7.25 (*s*, 1 H); 7.20–7.05 (*m*, 5 H); 5.92 (*d*, *J* = 8.4, 1 H); 4.91, 4.74 (2*d*, *J* = 12.8, total 2 H); 4.20–3.50 (*m*, 6 H); 3.22, 3.21 (2*s*, total 6 H); 1.80–1.68 (*s*, 3 H); 1.26, 1.23 (2*s*, total 6 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 163.8; 156.9; 151.6; 136.6; 135.9; 128.6; 128.2; 128.0; 127.8; 127.5; 127.3; 111.2; 100.0; 99.7; 82.1; 69.9; 67.7; 66.5; 62.0; 53.8; 49.7; 47.9; 17.7; 12.4. FAB-MS: 536.2 ([*M* + H]$^+$, 72), 504 (100). Anal. calc. for $C_{25}H_{33}N_3O_{10}$: C 56.07, H 6.21, N 7.85; found: C 55.75, H 6.07, N 7.33.

*Benzyl [5-(Dimethoxymethyl)-2,3-dimethoxy-2,3-dimethyl-7-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)hexahydro-5*H*-pyrano[3,4-*b*][1,4]dioxin-8-yl]carbamate* (**27**). To a soln. of **26** (400 mg, 0.76 mmol) in CH_2Cl_2 (20 ml) at 0° was added trichloroisocyanuric acid (177 mg, 0.76 mmol), and, after 5 min, TEMPO (12 mg, 0.08 mmol) was added. After stirring at 0° for 5 min, the soln. was stirred at r.t. for a further 10 min. The resulting orange soln. was filtered through a pad of *Celite* and washed with CH_2Cl_2. A sat. soln. of $NaHCO_3$ (60 ml) was then added, followed by brine (60 ml). The org. phase was extracted with CH_2Cl_2 (total 4 × 60 ml), and these fractions were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give the corresponding aldehyde (320 mg, 80%) as a white solid. To a portion of this aldehyde (300 mg, 0.56 mmol) in MeOH (10 ml) was added trimethyl orthoformate

(5 ml) and (+)-camphor-10-sulfonic acid monohydrate (40 mg, 0.17 mmol). The soln. was stirred at r.t. for 16 h, and then a 10% (*w*/*v*) aq. soln. of $NaHCO_3$ (20 ml) was added, followed by CH_2Cl_2 (30 ml). The org. phase was isolated, and extraction was repeated (total 4 × 30 ml of CH_2Cl_2). The org. fractions were combined, washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Purification by FC (5% MeOH/CH_2Cl_2) gave **27** (259 mg, 80%). White solid. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.39–7.18 (*m*, 6 H); 5.62, 5.38 (2*d*, *J* = 9.1, 9.5, total 1 H); 5.18, 5.02 (2*s*, total 2 H); 4.57 (*s*, 1 H); 4.00–3.50 (*m*, 4 H); 3.40 (*s*, 6 H); 3.30, 3.26 (2*s*, total 6 H); 1.80, 1.69 (2*s*, total 3 H); 1.30, 1.28 (2*s*, total 6 H). HR-ESI-MS (pos.): 668.2251 ($[M + Rb + 4\ H]^+$, $C_{27}H_{41}N_3O_{11}Rb^+$; calc. 668.1859).

*1-[8-Amino-5-(dimethoxymethyl)-2,3-dimethoxy-2,3-dimethyl-5*H*-hexahydropyrano[3,4-*b*][1,4]dioxin-7-yl]-5-methylpyrimidine-2,4(1*H,*3*H*)-dione* (**28**). To a soln. of **27** (200 mg, 0.38 mmol) in EtOH (5 ml) was added cyclohexene (3 ml) and then 10% Pd/C, and the mixture was heated at reflux for 90 min. The flask was cooled to r.t., the soln. was filtered through a plug of *Celite* and washed with excess MeOH. The filtrate was evaporated under reduced pressure and purified by FC (10% MeOH/CH_2Cl_2) to give **28** (104 mg, 69%). White solid. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.09 (*d*, *J* = 1.1, 1 H); 5.53 (*d*, *J* = 9.1, 1 H); 4.52 (*s*, 1 H); 3.83–3.75 (*m*, 2 H); 3.70–3.55 (*m*, 1 H); 3.45 (*s*, 6 H); 3.30, 3.29 (2*s*, total 6 H); 3.01 (*dd*, *J* = 9.5, 1 H); 1.88 (*d*, *J* = 1.1, 3 H); 1.32, 1.30 (2*s*, total 6 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 163.5; 151.0; 135.1; 112.0; 100.0; 99.7; 84.0; 73.4; 66.6; 56.3; 55.4; 54.2; 48.1; 17.7 (2 signals); 12.7. FAB-MS: 446.5 ($[M + H]^+$, 100), 414.4 (20). HR-ESI-MS (pos.) 468.2026 ($[M + Na]^+$, $C_{19}H_{31}N_3NaO_9^+$; calc. 468.1958).

*(6-Amino-1*H*-purin-9-yl)acetohydrazide* (**31**). To a suspension of **30** (632 mg, 2.86 mmol) in MeOH (20 ml) was added $NH_2NH_2 \cdot H_2O$ (1.39 ml, 28.6 mmol), and the mixture was stirred at r.t. for 16 h. The resulting precipitate was filtered, and washed with excess MeOH to give **31** (512 mg, 87%). White solid. M.p. > 285° (dec.). ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.46 (*s*, 1 H); 8.12 (*s*, 1 H); 8.08 (*s*, 1 H); 7.22 (*s*, 2 H); 4.80 (*s*, 2 H); 4.33 (*s*, 2 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 166.3; 156.3; 152.8; 150.1; 142.1; 118.8; 44.2. FAB-MS: 208.1 ($[M + H]^+$); HR-FAB-MS: 208.0948 ($C_7H_{10}N_7O^+$; calc. 208.0947). Anal. calc. for $C_7H_9N_7O \cdot 0.2\ CH_3OH$: C 40.49, H 4.62, N 45.90; found: C 40.57, H 4.37, N 46.13.

(2-Amino-6-oxo-1,6-dihydropurin-9-yl)acetohydrazide (**33**). To a soln. of **32** (150 mg, 0.56 mmol) in MeOH (50 ml) was added $NH_2NH_2 \cdot H_2O$ (200 mg, 7.10 mmol), and the mixture was refluxed for 18 h. The precipitated product was filtered off, washed with MeOH (20 ml), and dried under vacuum to give pure **33** (99 mg, 79%). White solid. M.p. > 350° (dec.). ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.31 (br. *s*, 1 H); 7.61 (*s*, 1 H); 6.42 (br. *s*, 2 H); 4.59 (*s*, 2 H); 4.29 (br. *s*, 2 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 166.4; 157.2; 154.0; 152.0; 138.7; 116.6; 44.0. ESI-MS (pos.): 224.4 ($[M + H]^+$). HR-ESI-MS (pos.): 224.0898 ($[M + H]^+$, $C_7H_9N_7O_2^+$; calc. 224.0818).

*(5-Methyl-2,4-dioxo-1*H*,3*H*-pyrimidin-1-yl)acetohydrazide* (**34**). To a soln. of *(thymin-1-yl)acetic acid* (**1**; 1.00 g, 5.43 mmol) and NH_2NHCOO^tBu (0.72 g, 5.43 mmol) in DMF (20 ml) was added EDC (1.04 g, 5.43 mmol), and the soln. was stirred at r.t. for 12 h. The soln. was then evaporated under reduced pressure, and AcOEt (30 ml) and H_2O (30 ml) were added, and the org. phase isolated. Extraction was repeated (total 3 × 30 ml of AcOEt), and the org. fractions were combined, washed with brine, and dried (Na_2SO_4). Filtration and evaporation under reduced pressure gave a white solid to which was added 1M aq. HCl (20 ml), and the soln. was stirred at r.t. for 12 h. The soln. was evaporated under reduced pressure to give **34** (1.08 g, 62%). White solid. M.p. > 355° (dec.). ^{1}H-NMR (200 MHz, D_2O): 7.43 (*s*, 1 H); 4.53 (*s*, 2 H); 1.87 (*s*, 3 H). ^{13}C-NMR (50 MHz, D_2O): 168.7; 167.7; 152.9; 143.6; 111.9; 49.8; 12.0. FAB-MS: 199 ($[M + H]^+$, 100). HR-FAB-MS: 199.0844 ($C_7H_{11}N_4O_3^+$; calc. 199.0831). Anal. calc. for $C_7H_{10}N_4O_3 \cdot 0.5$ HCl: C 38.94, H 4.90, N 25.95; found: C 39.01, H 4.87, N 25.45.

tert-*Butyl* N′*-[2-({[(4-Benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(*2H*)-yl)acetyl]hydrazinecarboxylate* (**35**). To a soln. of *2-{*N^4*-[(Benzyloxy)carbonyl]cytosin-1-yl}acetic acid* (**29**; 1.20 g, 3.96 mmol) and NH_2NHCOO^tBu (0.52 g, 3.96 mmol) in DMF (20 ml) was added EDC (0.76 g, 3.96 mmol), and the soln. was stirred at r.t. for 12 h, whereupon the solvent was evaporated. AcOEt (30 ml) and H_2O (30 ml) were then added, and the org. phase was extracted (total 3 × 30 ml of AcOEt). The org. fractions were combined, washed with brine, and dried (Na_2SO_4). Filtration and evaporation under reduced pressure gave pure **35** (1.07 g, 65%). White solid. M.p. 165–167°. ^{1}H-NMR (400 MHz, CD_3OD): 7.96 (*d*, *J* = 7.3, 1 H); 7.30–7.50 (*m*, 6 H); 5.25 (*s*, 2 H); 4.65 (*s*, 2 H); 1.49 (*s*, 9 H). ^{13}C-NMR (101 MHz, CD_3OD): 167.7; 164.1; 157.0; 156.3; 153.1; 150.1; 135.8; 128.2; 127.9; 95.4; 80.7; 67.2; 50.2; 27.1; FAB-MS: (440, $[M + Na]^+$, 18), 418 ($[M + H]^+$, 100), 362 (37). HR-FAB-MS: 418.1722, ($C_{19}H_{24}N_5O_6^+$; calc. 418.1727).

*2-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)acetohydrazide* (**36**). To a soln. of **35** (150 mg, 0.39 mmol) in EtOH (5 ml) was added cyclohexene (2 ml) and 10% Pd/C (150 mg), and the soln. was heated at reflux for 2 h. The soln. was cooled to r.t., filtered through a pad of *Celite*, which was washed with excess MeOH. After evaporation under reduced pressure, the intermediate product was purified by FC (SiO_2; MeOH/CH_2Cl_2 1:4) to give a white solid. To this solid was added 1M aq. HCl (5 ml), and the soln. was stirred at r.t. for 12 h. Evaporation under reduced pressure gave the dihydrochloride salt of **36** (71 mg, 71%). White solid. M.p. >270° (dec.). ^{1}H-NMR (200 MHz, D_2O): 7.84 (*d*, *J* = 7.8, 1 H); 6.23 (*d*, *J* = 7.8, 1 H); 4.72 (*s*, 2 H). ^{13}C-NMR (101 MHz, D_2O): 167.3; 160.3; 150.4; 149.5; 95.7; 50.4. ESI-MS (pos.) 367.1 ($[2M+H]^+$), 184.0 ($[M+H]^+$). HR-ESI-MS (pos.) 184.0851 ($C_6H_{10}N_5O_2^+$; calc. 184.0829). Anal. calc. for $C_6H_9N_5O_2 \cdot 2.1$ HCl: C 27.74, H 4.31, N 26.95; found: C 28.04, H 4.11, N 26.37.

N-*(2,2-Dimethoxyethyl)-2-(5-methyl-2,4-dioxo-(1*H*,3*H*-pyrimidin-1-yl)acetamide* (**37**). To a soln. of aminoacetaldehyde dimethyl acetal (1.00 ml, 9.30 mmol) in DMF (10 ml) was added EDC (2.13 g, 11.2 mmol) and **1** (2.05 g, 11.2 mmol), and the cloudy soln. was stirred for 12 h. The resulting homogeneous soln. was then evaporated under reduced pressure, and AcOEt (20 ml) and H_2O (20 ml) were added. The org. phase was isolated, and, after further extraction with AcOEt (4 × 20 ml), the org. phases were combined, dried (Na_2SO_4), and evaporated under reduced pressure. Purification by FC (SiO_2; MeOH/CH_2Cl_2 1:9) gave **37** (0.49 g, 20%). White solid. M.p. 191–192°. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.08 (*d*, *J* = 1.1, 1 H); 6.34 (br., 1 H); 4.40 (*t*, *J* = 5.1, 1 H); 4.30 (*s*, 2 H); 3.44 (*d*, *J* = 5.5, 2 H); 3.40 (*s*, 6 H); 1.93 (*d*, *J* = 1.1, 3 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 167.2; 164.4; 151.5; 141.2; 111.8; 102.9; 55.2; 51.2; 41.8; 13.0. FAB-MS: 272 ($[M+H]^+$, 75), 240 (100). Anal. calc. for $C_{11}H_{17}N_3O_5$: C 48.70, H 6.32, N 15.49; found: C 48.75, H 6.28, N 15.39.

*Benzyl (1-{[(2,2-Dimethoxyethyl)carbamoyl]methyl}-2-oxopyrimidin-4(2*H*)-yl)carbamate* (**38**). To a soln. of *2-{N^4-[(Benzyloxy)carbonyl]cytosin-1-yl}acetic acid* (**29**; 1.60 g, 5.28 mmol) and EDC (1.01 g, 5.28 mmol) in DMF (30 ml) was added aminoacetaldehyde dimethyl acetal (0.38 ml, 3.52 mmol), and the soln. was stirred at r.t. for 12 h. The soln. was evaporated under reduced pressure to give a white solid, to which was added AcOEt (30 ml) and H_2O (60 ml). The resulting insoluble material was filtered and washed again with AcOEt (30 ml) and then CH_2Cl_2 (30 ml), and then dried under reduced pressure to give **38** (1.22 g, 92%). White solid. M.p. 210–212°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.33 (*t*, *J* = 5.7, 1 H); 7.99 (*d*, *J* = 7.3, 1 H); 7.33–7.45 (*m*, 5 H); 7.01 (*d*, *J* = 7.3, 1 H); 5.20 (*s*, 2 H); 4.49 (*s*, 2 H); 4.36 (*t*, *J* = 5.5, 1 H); 3.29 (*s*, 6 H); 3.21 (*dd*, *J* = 5.6, 5.6, 2 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 167.4; 163.5; 155.5; 153.6; 151.5; 136.4; 128.9; 128.6; 128.4; 102.4; 94.1; 66.9; 53.8; 51.7. FAB-MS: 391 ($[M+H]^+$, 100), 359 (50). HR-FAB-MS: 391.1614 ($C_{18}H_{23}N_4O_6^+$; calc. 391.1618). Anal. calc. for $C_{18}H_{22}N_4O_6$: C 55.38, H 5.68, N 14.35; found: C 55.23, H 5.67, N 14.50.

*2-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-*N*-(2,2-dimethoxyethyl)acetamide* (**39**). To a suspension of **38** (1.08 g, 2.89 mmol) in EtOH (40 ml) was added cyclohexene (10 ml) and 10% (*w*/*v*) Pd/C (0.54 g), and the mixture was heated at reflux for 1 h. The soln. was then cooled to r.t., filtered through a pad of *Celite*, which was subsequently washed with MeOH (50 ml). Evaporation under reduced pressure, followed by purification by FC (SiO_2; gradient elution 10 → 15% MeOH/CH_2Cl_2), gave **39** (0.70 g, 95%). White solid. M.p. 209–211°. ^{1}H-NMR (400 MHz, CD_3OD): 7.62 (*d*, *J* = 7.3, 1 H); 5.94 (*d*, *J* = 7.3, 1 H); 4.49 (*s*, 2 H); 4.44 (*t*, *J* = 5.6, 1 H); 3.41 (*s*, 6 H); 3.36 (*d*, *J* = 5.6, 2 H). ^{13}C-NMR (101 MHz, CD_3OD): 169.8; 166.8; 157.3; 149.0; 103.8; 95.7; 54.6; 52.4; 42.3. FAB-MS: 279.1 ($[M+Na]^+$, 17), 257.1 ($[M+H]$, 100), 225 (58), 152 (62). HR-FAB-MS: 257.1258 ($C_{10}H_{17}N_4O_4^+$; calc. 257.1250).

*1-(2-{[(2,2-Dimethoxyethyl)methyl]amino}ethyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione* (**40**). To a mixture of *2-(thymin-1-yl)ethanal hydrate* (**20**; 400 mg, 2.15 mmol) and 2-(methylamino)acetaldehyde dimethyl acetal (255 mg, 2.15 mmol) in MeOH (10 ml) was added $NaCNBH_3$ (206 mg, 3.2 mmol), and the mixture was stirred for 48 h at r.t. The reaction was quenched with H_2O (1 ml), and the solvent was evaporated to dryness. $CHCl_3$ (150 ml) was added, the soln. was washed with sat. $NaHCO_3$ soln. and brine, and dried (Na_2SO_4). Evaporation of the solvent gave a crude product which was purified by FC (SiO_2; MeOH/CH_2Cl_2 1:19) to give **40** (400 mg 62%). White solid. M.p. 75–76°. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.65 (br. *s*, 1 H); 7.22 (*s*, 1 H); 4.41 (*t*, *J* = 8.0, 1 H); 3.78 (*t*, *J* = 4.0, 2 H); 3.37 (*s*, 6 H); 2.72 (*t*, *J* = 8.0, 2 H); 2.59 (*d*, *J* = 8.0, 2 H); 2.37 (*s*, 3 H); 1.94 (*s*, 3 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 164.4; 151.0; 142.0; 109.4; 103.0; 59.1; 56.5; 53.7; 46.0; 43.6; 12.2. ESI-MS (pos.): 272.1 ($[M+H]^+$). Anal. calc. for $C_{12}H_{21}N_3O_4$: C 53.12, H 7.80, N 15.49; found: C 52.83, H 7.79, N 15.39.

*9-(2-{[(2,2-Dimethoxyethyl)methyl]amino}ethyl)-9*H*-purin-6-amine* (**41**). To a mixture of *2-(adenin-9-yl)ethanal hydrate* (**8**; 200 mg, 0.86 mmol) and 2-methylamino)acetaldehyde dimethyl acetal (110 mg, 0.86 mmol) in MeOH (10 ml) was added $NaCNBH_3$ (80 mg, 1.29 mmol), and the mixture was stirred for 2 days at r.t. The reaction was quenched with H_2O (1 ml), and the solvent was evaporated to dryness. $CHCl_3$ (150 ml) was added, the soln. was washed with sat. $NaHCO_3$ and brine, and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give a crude product, which was purified by FC (SiO_2; MeOH/CH_2Cl_2 1:49) to give **41** (160 mg, 66%). White solid. M.p. 130–131°. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.38 (*s*, 1 H); 8.06 (*s*, 1 H); 5.68 (br. *s*, 2 H); 4.39 (*t*, *J* = 4.0, 1 H); 4.31 (*t*, *J* = 4.0, 2 H); 3.33 (*s*, 6 H); 2.92 (*t*, *J* = 8.0, 2 H); 2.64 (*d*, *J* = 4.0, 2 H); 2.41 (*s*, 3 H). ^{13}C-NMR (101 MHz, $CDCl_3$): 155.3; 152.7; 150.0; 141.7; 119.4; 103.1; 59.2; 57.1; 53.7; 43.3; 41.6. ESI-MS: 281.1 ($[M+H]^+$). Anal. calc. for $C_{12}H_{20}N_6O_2 \cdot 0.05\ H_2O$: C 51.25, H 7.20, N 29.88; found: C 51.92, H 7.16, N, 29.79.

*5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-carbohydrazide* (**46**). To a soln. of *uridine-5′-methyl ester* (**43**; 200 mg, 0.73 mg) in MeOH (150 ml) was added $NH_2NH_2 \cdot H_2O$ (60 µl), and the soln. was stirred at r.t. overnight. The solvent was evaporated, and the crude product was purified by FC (RP-C_{18} silica gel; MeOH/H_2O) to give **46** (55 mg, 27). Light yellow solid (hygroscopic, unstable at r.t.). M.p. 106–108°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.63 (br. *s*, 1 H); 8.33 (*d*, *J* = 8.0, 1 H); 5.88 (*d*, *J* = 6.0, 1 H); 5.70 (*d*, *J* = 8.0, 1 H); 5.43 (*m*, 1 H); 4.43 (*m*, 1 H); 4.23 (br. *s*, 2 H); 3.36 (*d*, *J* = 4.0, 1 H). ^{13}C-NMR (101 MHz D_2O + *t*BuOH): 169.7; 166.4; 151.8; 142.7; 102.4; 90.7; 81.8; 72.5; 72.2. FAB-MS: 272.8 (M^+). HR-FAB-MS: 295.1029 ($C_9H_{12}N_4NaO_6^+$; calc. 295.2068).

*5-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-carbohydrazide* (**47**). To a soln. of *cytosine-5′-methyl ester* (**44**; 100 mg, 0.36 mmol) in EtOH (20 ml) was added $NH_2NH_2 \cdot H_2O$ (60 µl), and the soln. was stirred at r.t. for 48 h. The mixture was then concentrated under reduced pressure, and the crude product was purified by FC (RP-C_{18} silica gel; H_2O) to give **47** (20 mg, 20). Light yellow solid (hygroscopic, unstable at r.t.). M.p. 185–187°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.67 (br. *s*, 1 H); 8.17 (*d*, *J* = 7.2, 1 H); 7.21–7.17 (*m*, 2 H); 5.81 (*d*, *J* = 4.8, 1 H); 5.74 (*d*, *J* = 7.6, 1 H); 5.45 (*m*, 2 H); 4.40 (br. *s*, 2 H); 4.19 (*d*, *J* = 3.2, 1 H); 4.10 (*m*, 1 H); 3.99 (*m*, 1 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 169.1; 166.0; 155.9; 142.7; 94.5; 90.6; 82.0; 73.7; 73.0. ESI-MS (pos.): 272.1 (M^+). HR-FAB-MS: 272.0995 ($C_9H_{13}N_4O_6^+$; calc. 272.2302).

*5-(6-Aminopurin-9(1*H*)-yl)-3,4-dihydroxy*-N-*(2,2-dimethoxyethyl)tetrahydrofuran-2-carboxamide* (**49**). A soln. of *adenosine-5′-methyl ester* (**42**; 100 mg, 0.33 mmol) in aminoacetaldehyde dimethyl acetal (4 ml) was heated at 60–70° for 24 h. The mixture was then evaporated to dryness, and the crude product was purified by FC (SiO_2; MeOH/CH_2Cl_2 1:9) to give **49** (65 mg, 52%). White solid. M.p. 160–161°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.08 (*t*, *J* = 8.0, 1 H); 8.37 (*s*, 1 H); 8.21 (*s*, 1 H); 7.43 (br. *s*, 2 H); 5.99 (*d*, *J* = 7.2, 1 H); 5.78 (*d*, *J* = 4.0, 1 H); 5.59 (*d*, *J* = 6.6, 1 H); 4.61–4.56 (*m*, 1 H); 4.42 (*t*, *J* = 5.6, 1 H); 4.36 (*s*, 1 H); 4.15 (*t*, *J* = 3.6, 1 H); 3.32 (*s*, 3 H); 3.25 (*s*, 3 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 170.3; 156.7; 153.0; 149.3; 141.0; 120.0; 102.4; 88.1; 85.0; 73.7; 72.4; 53.9; 53.7; 40.7. ESI-MS: 369.1538 ($[M+H]^+$, $C_{14}H_{21}N_6O_6^+$; calc. 369.1523).

N-*(2,2-Dimethoxyethyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-carboxamide* (**50**). A soln. of *uridine-5′-methyl ester* (**43**; 300 mg, 1.10 mmol) in aminoacetaldehyde dimethyl acetal (4 ml) was heated at 60–70° for 2 h. The mixture was then evaporated to dryness, and the crude product was purified by FC (SiO_2; MeOH/CH_2Cl_2 1:9) to give **50** (330 mg, 87%). White solid. M.p. 184–185°. ^{1}H-NMR (400 MHz, (D_6)DMSO): 11.33 (br. *s*, 1 H); 8.42 (*t*, *J* = 5.6, 1 H); 8.24 (*d*, *J* = 8.4, 1 H); 5.90 (*d*, *J* = 6.4, 1 H); 5.70 (*d*, *J* = 8.0, 1 H); 5.57 (*s*, 2 H); 4.42 (*t*, *J* = 5.6, 1 H); 4.34 (*s*, 1 H); 4.17 (*d*, *J* = 4.8, 1 H); 3.99 (*m*, 1 H); 3.38 (*s*, 2 H); 3.27 (*s*, 6 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 170.6; 163.5; 151.4; 141.6; 102.5; 102.0; 88.2; 83.4; 73.5; 73.4; 53.7; 53.6. FAB-MS: 368.1 ($[M+Na]^+$, 100). Anal. calc. for $C_{13}H_{19}N_3O_8$: C 45.22, H 5.55, N 12.17; found: C 44.81, H 5.26, N 11.97.

REFERENCES

[1] J.-M. Lehn, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4763; J.-M. Lehn, *Science* **2002**, *295*, 2400; J.-M. Lehn, *Chem. Soc. Rev.* **2007**, *36*, 151.

[2] a) J.-M. Lehn, *Chem.–Eur. J.* **1999**, *5*, 2455; b) O. Ramström, J.-M. Lehn, *Nat. Rev. Drug Discov.* **2002**, *1*, 26; c) P. T. Corbett, J. Leclaire, J. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652; d) I. Huc, R. Nguyen, *Comb. Chem. High Throughput Screen.* **2001**, *4*, 53.

[3] a) I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2106; b) O. Ramström, J.-M. Lehn, *ChemBioChem* **2000**, *1*, 41; c) T. Bunyapaiboonsri, O. Ramström, S. Lohmann, J.-M. Lehn, L. Peng, M. Goeldner, *ChemBioChem* **2001**, *2*, 438; d) T. Bunyapaiboonsri, H. Ramström, O. Ramström, J. Haiech, J.-M. Lehn, *J. Med. Chem.* **2003**, *46*, 5803; e) O. Ramström, S. Lohmann, T. Bunyapaiboonsri, J.-M. Lehn, *Chem.–Eur. J.* **2004**, *10*, 1711; f) M. Hochgürtel, H. Kroth, D. Piecha, M. W. Hofmann, C. Nicolau, S. Krause, O. Schaaf, G. Sonnenmoser, A. V. Eliseev, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3382; g) M. Hochgürtel, R. Biesinger, H. Kroth, D. Piecha, M. W. Hofmann, S. Krause, O. Schaaf, C. Nicolau, A. V. Eliseev, *J. Med. Chem.* **2003**, *46*, 356; h) S. Gerber-Lemaire, F. Popowycz, E. Rodríguez-García, A. T. C. Asenjo, I. Robina, P. Vogel, *ChemBioChem* **2002**, *3*, 466; i) S. Zameo, B. Vauzeilles, J.-M. Beau, *Angew. Chem., Int. Ed.* **2005**, *44*, 965.

[4] a) J.-M. Lehn, *Polym. Int.* **2002**, *51*, 825; b) W. G. Skene, J.-M. Lehn, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8270; c) J.-M. Lehn, *Prog. Polym. Sci.* **2005**, *30*, 814.

[5] H. Otsuka, K. Aotani, Y. Higaki, A. Takahara, *Chem. Commun.* **2002**, 2838; H. Otsuka, K. Aotani, Y. Higaki, A. Takahara, *J. Am. Chem. Soc.* **2003**, *125*, 4064; T. Ono, T. Nobori, J.-M. Lehn, *Chem. Commun.* **2005**, 1522.

[6] a) N. Sreenivasachary, D. T. Hickman, J.-M. Lehn, unpublished results; b) N. Sreenivasachary, D. T. Hickman, D. Sarazin, J.-M. Lehn, *Chem.–Eur. J.* **2006**, *12*, 8531.

[7] M. Di Marzo, S. Lohmann, J.-M. Lehn, unpublished results.

[8] Y. Ruff, J.-M. Lehn, unpublished results.

[9] D. N. Bolon, C. A. Voigt, S. L. Mayo, *Curr. Opin. Chem. Biol.* **2002**, *6*, 125; G. N. Tew, D. Liu, B. Chen, R. J. Doerksen, J. Kaplan, P. J. Karroll, M. L. Klein, W. F. DeGrado, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5110; M. A. Dwyer, L. L. Looger, H. W. Hellinga, *Science* **2004**, *304*, 1967.

[10] H. Kobayashi, M. Amaike, K. Koumoto, S. Shinkai, *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1311.

[11] K. Oh, K.-S. Jeong, J. S. Moore, *Nature* **2001**, *414*, 889; D. Zhao, J. S. Moore, *J. Am. Chem. Soc.* **2002**, *124*, 9996.

[12] a) N. Giuseppone, J.-L. Schmitt, J.-M. Lehn, *Angew. Chem., Int. Ed.* **2004**, *43*, 4902; b) N. Giuseppone, J.-M. Lehn, *J. Am. Chem. Soc.* **2004**, *126*, 11448; c) N. Sreenivasachary, J.-M. Lehn, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5938.

[13] S. Otto, R. L. E. Furlan, J. K. M. Sanders, *J. Am. Chem. Soc.* **2000**, *122*, 12063; S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Science* **2002**, *297*, 590.

[14] R. Larsson, Z. Pei, O. Ramström, *Angew. Chem., Int. Ed.* **2004**, *43*, 3716.

[15] B. Shi, M. F. Greaney, *Chem. Commun.* **2005**, 886.

[16] K. C. Nicolaou, R. Hughes, S. K. Cho, N. Winssinger, C. Smethurst, H. Labischinski, R. Endermann, *Angew. Chem., Int. Ed.* **2000**, *39*, 3823.

[17] R. J. Lins, S. L. Flitsch, N. J. Turner, E. Irving, S. A. Brown, *Angew. Chem., Int. Ed.* **2002**, *41*, 3405.

[18] a) R. L. E. Furlan, Y.-F. Ng, S. Otto, J. K. M. Sanders, *J. Am. Chem. Soc.* **2001**, *123*, 8876; b) S. L. Roberts, R. L. E. Furlan, G. R. L. Cousins, J. K. M. Sanders, *Chem. Commun.* **2002**, 938; c) R. Nguyen, I. Huc, *Chem. Commun.* **2003**, 942.

[19] a) S. Lohmann, T. Bunyapaiboonsri, O. Ramström, J.-M. Lehn, unpublished results; b) S. Lohmann, Ph.D. Thesis, l'Université Louis Pasteur, Strasbourg, 2003.

[20] A. De Mesmaeker, A. Waldner, J. Lebreton, P. Hoffman, V. Fritsch, R. M. Wolf, S. M. Freier, *Angew. Chem., Int. Ed.* **1994**, *33*, 226; A. De Mesmaeker, C. Jouanno, R. M. Wolf, S. Wendeborn, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 447; D. D. Weller, D. T. Daly, W. K. Olson, J. E. Summerton, *J. Org. Chem.* **1991**, *56*, 6000.

[21] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497; P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624.

[22] J. Summerton, D. Weller, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 187; O. Chakhmakhcheva, M. Andrianov, A. Buryakova, M. Choob, V. Efimov, *Nucleosides Nucleotides* **1999**, *18*, 1427; T. Vilaivan, G. Lowe, *J. Am. Chem. Soc.* **2002**, *124*, 9326.

[23] R. A. Goodnow Jr., A.-R. Richou, S. Tam, *Tetrahedron Lett.* **1997**, *38*, 3195; R. A. Goodnow Jr., S. Tam, D. L. Pruess, W. W. McComas, *Tetrahedron Lett.* **1997**, *38*, 3199.
[24] a) K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen, O. Buchardt, *J. Org. Chem.* **1994**, *59*, 5767; b) P. J. Finn, N. J. Gibson, R. Fallon, A. Hamilton, T. Brown, *Nucleic Acids Res.* **1996**, *24*, 3357; c) D. W. Will, G. Breipohl, D. Langner, J. Knolle, E. Uhlmann, *Tetrahedron* **1995**, *51*, 12069.
[25] B. Hyrup, M. Egholm, O. Buchardt, P. E. Nielsen, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083.
[26] X. Li, Z.-Y. J. Zhan, R. Knipe, D. G. Lynn, *J. Am. Chem. Soc.* **2002**, *124*, 746; X. Li, D. G. Lynn, *Angew. Chem., Int. Ed.* **2002**, *41*, 4567; D. M. Rosenbaum, D. R. Liu, *J. Am. Chem. Soc.* **2003**, *125*, 13924.
[27] C. Godoy-Alacantor, A. K. Yatsimirsky, J.-M. Lehn, *J. Phys. Org. Chem.* **2005**, *18*, 979.
[28] H. Sugihara, H. Fukushi, T. Miyawaki, Y. Imai, Z. Terashita, M. Kawamura, Y. Fujisawa, S. Kita, *J. Med. Chem.* **1998**, *41*, 489.
[29] M. T. Doel, A. S. Jones, N. Taylor, *Tetrahedron Lett.* **1969**, *27*, 2285; A. P. Martinez, W. W. Lee, *J. Org. Chem.* **1965**, *30*, 317; A. J. H. Nollet, C. M. Hutino, U. K. Pandit, *Tetrahedron* **1969**, *25*, 5971.
[30] J. Yano, T. Ohgi, K. Ishiyama, H. Tomi, PCT Int. Pat. Appl. WO9118898, 1991.
[31] R. S. Strong, P. H. Gross, H. K. Zimmermann, *Liebigs Ann. Chem.* **1966**, *692*, 215.
[32] S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, J. F. Stoddart, *Angew. Chem., Int. Ed.* **2002**, *41*, 899.
[33] T. Kofoed, H. F. Hansen, H. Ørum, T. Koch, *J. Pept. Sci.* **2001**, *7*, 402.
[34] G. Breipohl, D. W. Will, A. Peyman, E. Uhlmann, *Tetrahedron* **1997**, *53*, 14671.
[35] K. A. Jacobsen, M. Ohno, H. T. Duong, S.-K. Kim, S. Tchilibon, M. Česnek, A. Holý, Z.-G. Gao, *Chem. Biol.* **2005**, *12*, 237.
[36] S. F. Wnuk, S. Liu, C.-S. Yuan, R. T. Borchardt, M. J. Robins, *J. Med. Chem.* **1996**, *39*, 4162.
[37] K. E. Norris, O. Manscher, K. Brunfeldt, J. B. Petersen, *Nucleic Acids Res.* **1975**, *7*, 1093.

Received October 4, 2007