Solid-Phase Synthesis of Dipeptide-Conjugated Nucleosides and Their Interaction with RNA

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Dipeptide-conjugated nucleosides were efficiently synthesized from the intermediates of 3′-amino-3′-deoxy-nucleosides by using the solid-phase synthetic strategy with HOBt/HBTU (1-hydroxy-1*H*-benzotriazole/2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoroborate) as the coupling reagents (*Schemes 1–3*). CD Spectra and thermal melting studies showed that the synthesized hydrophobic dipeptide–thymidine and –uridine derivatives **8a–8d**, **13a–d**, and **18** had a mild affinity with the polyA·polyU duplex and could induce the change of RNA conformation. The results also implied that the interaction of conjugates with RNA might be related to the sugar pucker conformation of the nucleoside.

Introduction. – The functional and structural diversities of RNA provide numerous opportunities for academic researchers and pharmaceutical industry to develop small molecules to target specific RNA for treating a variety of diseases, such as bacteria or virus infections [1]. The RNA secondary structure of base pairing is more conservative than its primary sequence, so the potential for slower development of drug resistance against small molecules is one of the advantages of targeting RNA over traditional protein targets. Aminoglycosides, a class of structurally diverse aminocyclitols with potent antibiotic and antiviral activities are intensively and well-studied RNA binders. They can selectively and stoichiometrically bind with functional RNA motifs and disrupt the protein–RNA, RNP–RNA, or RNA–RNA interaction [2]. On the basis of the study of RNA in the presence of aminoglycosides and other small molecules, the interactions of RNA with small molecules are affected by the distribution of charged, aromatic, and H-bonding groups of a relatively rigid scaffold [1]. Considering the interaction of protein–RNA, the α-helix conformation of the protein provides a scaffold for H-bonding with RNA bases, the β-sheet form is suitable for binding aromatic groups with unstacked bases, and the negative phosphodiester moiety of RNA supplies a function for the electrostatic interaction. Furthermore, the structure of RNA is dynamic, and the conformational change can be induced by a small molecule [2]. The structural and functional features of RNA stimulated our enthusiasm to find more-selective and potent small molecules, and the adoption of virtual screening, surface plasmon resonance (SPR), and other technologies also speeded up the process [3].

Our previous findings indicated that aminoglycosyl-nucleosides could bind to RNA with high affinity [4]. Besides that the configuration of the glycosyl moiety could affect their interaction, the heterocyclic-base moiety also showed an effect on the base stacking of the RNA duplex. Peptides have a relatively flexible conformation as compared to a glycosyl moiety. The diverse functional groups of a peptide, such as

HCA

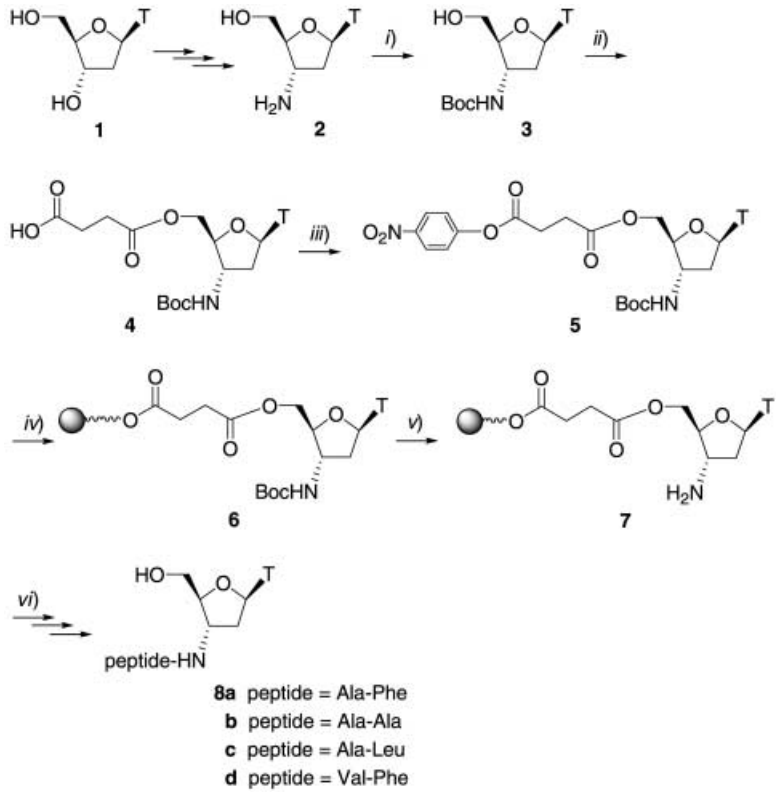
HELVETICA CHIMICA ACTA
Verlag Helvetica Chimica Acta AG
Druck: K. Triltsch, Print und digitale Medien GmbH,
D-97199 Ochsenfurt-Hohestadt

hydrophobic, basic, or acidic groups, could provide multiform H-bonding or electrostatic interactions with RNA. Therefore, a small-peptide-conjugated nucleoside could be expected to exhibit affinity with an RNA duplex and interrupt RNA function, and selectivity could be achieved by choosing an appropriate nucleoside and peptide. Actually, many naturally occurring or synthesized peptide-conjugated nucleosides have shown antibacterial or antiviral activities [5]. In the early years, *Robins* and co-workers indicated that some natural nucleoside–peptides were inhibitors of protein synthesis, and peptides bound to DNA may play a role in protein synthesis by acting as 'derepressors' of structural genes [6]. Puromycin, an amino acid conjugated nucleoside, resembles the structure of the 3′-end of aminoacyl-tRNA which can bind to the ribosomal A-site [7]. On the other hand, many nucleoside or nucleoside-mimetic drugs have been conjugated to the structural and functional peptides to improve their selectivity or efficiency and to overcome the problems of side effects [8]. In this paper, we present a concise method for the synthesis of peptide-conjugated nucleosides. This solid-phase peptide-synthesis strategy can be modified and used for constructing a compound library. Our results also show that the dipeptide-conjugated thymidines and uridines could bind to RNA and change the RNA conformation slightly.

Results and Discussion. – *Chemical Syntheses of the Dipeptide-Conjugated Nucleosides.* As the starting compound for the synthesis of thymidine derivatives, 3′-amino-3′-deoxythymidines were used. The 5′-OH of the nucleoside was used for the attachment to the solid support. To improve the efficiency of the synthesis, a succinyl moiety was used as a spacer connecting the solid support and the nucleoside (*Scheme 1*). Thus 3′-amino-3′-deoxythymidine (**2**) was synthesized in six steps in 37.2% overall yield from thymidine (**1**) [9]. The amino group of **2** was protected by the (*tert*-butoxy)carbonyl (Boc) group [10] (→**3**) and then converted to ester **4** by treatment with succinic anhydride in the presence of *N*,*N*-dimethylpyridin-4-amine (DMAP) under Ar [11]. The free COOH group of **4** was activated as 4-nitrophenyl ester (→**5**) for the efficient coupling with the aminomethyl (AM) resin [12] (→**6**). After removal of the Boc group with 33% CF_3COOH in CH_2Cl_2, an amount of 170 μmol/g of the nucleoside-loaded resin **7** was obtained, which was treated with amino acids by the solid-phase peptide-synthesis strategy to give the dipeptide-conjugated thymidines **8a**–**d**. The solid-phase synthesis of these dipeptide-conjugated nucleosides was carried out by means of standard Boc strategy, using HOBt (1-hydroxy-1*H*-benzotriazole) and HBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) as the coupling reagents in the presence of diisopropylethylamine ($^i Pr_2EtN$) [13]. Several kinds of nonpolar amino acids were chosen to compose the dipeptides. The dipeptide-conjugated thymidines **8a**–**d** were released from the resin with ammonia, purified by column chromatography (silica gel), and identified by ESI-TOF-MS and ^{1}H-NMR.

Dipeptide-conjugated xylofuranosyl-thymine was synthesized in the same way as described for the synthesis of dipeptide-conjugated thymidines (*Scheme 2*). The intermediate (3′-amino-2′,3′-dideoxy-β-D-xylofuranosyl)thymine **12** was synthesized starting from thymidine (**1**). The 5′-*O*-tritylthymidine 3′-(methanesulfonate) (**9**) was obtained in an overall yield of 68.2% after thymidine had been successively treated with trityl chloride and methanesulfonyl chloride [9]. Treatment of **9** with sodium azide under Ar gave **10** in 71.0% yield. After deprotection of **10** by treatment with 80%

Scheme 1

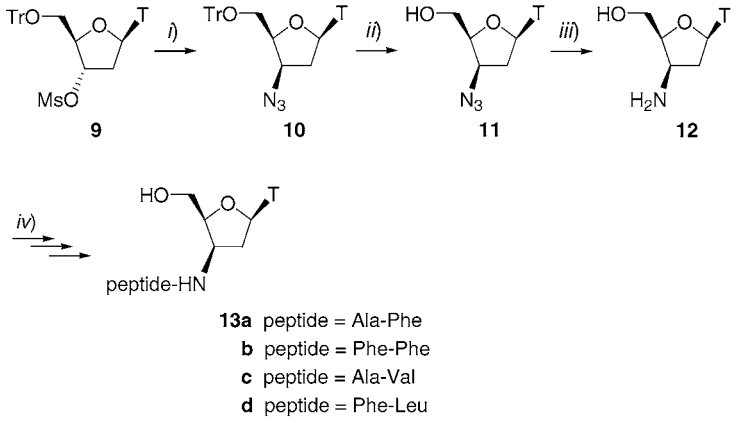


i) Di(*tert*-butyl) dicarbonate, EtOH. *ii*) Succinic anhydride, pyridine, DMAP. *iii*) 4-Nitrophenol, pyridine, DCC. *iv*) AM resin, DMF. *v*) 33% CF_3COOH in CH_2Cl_2. *vi*) Standard peptide synthesis.

AcOH ($\rightarrow$ **11**), catalyzed hydrogenation gave (3′-amino-2′,3′-dideoxy-β-D-xylofuranosyl)thymine (**12**). Compound **12** was bound to the AM resin *via* a succinyl linker in the same way as described for the synthesis of **7**; an amount of 110 μmol/g of the nucleoside-loaded resin was obtained. Subsequent standard solide-phase synthesis as described for **8a**–**d** yielded the dipeptide-conjugated nucleosides **13a**–**d**, which were similarly identified (see *Exper. Part*).

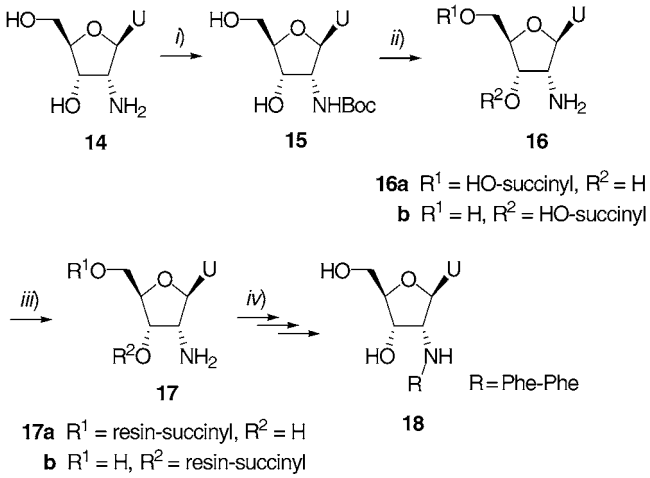
Regarding the synthesis of resin-bound 2′-amino-2′-deoxyuridine **17**, a similar sequence starting from **14** was performed (*Scheme 3*). Since both the 3′-OH and 5′-OH groups were active towards esterification, two compounds **16a** and **16b** were obtained, when **15** was treated with succinic anhydride. The FAB-MS showed only a monoester peak at 444.1 ($[M+1]^+$); no diester was observed. The ^{1}H-NMR spectrum established that a ratio of *ca.* 1:1 of the 5′- and 3′-monoester **16a** and **16b**, respectively, was obtained. These monoesters could not be easily separated by column chromatography (silica gel); thus, they were coupled with the AM resin as a mixture ($\rightarrow$ **17a**/**17b**). A total amount of 87 μmol/g of nucleoside was loaded on the resin. The subsequent solid-

Scheme 2



i) NaN_3, DMF, Ar, 100°. *ii*) 80% AcOH, r.t. *iii*) Pd/C, H_2. *iv*) Standard peptide synthesis.

Scheme 3



i) Di(*tert*-butyl) dicarbonate, MeCN, Et_3N. *ii*) Succinic anhydride, pyridine, DMAP. *iii*) 1. 4-nitrophenol, pyridine, DCC; 2. AM resin, DMF; 3. 33% CF_3COOH in CH_2Cl_2. *iv*) Standard peptide synthesis.

phase peptide synthesis (→**18**) indicated that the solid-support-linkage mode had almost no influence on the synthesis efficiency.

Interaction of Dipeptide-Conjugated Nucleosides with polyA·polyU. Thermal melting analysis and circular dichroism (CD) were used to investigate the interaction of the dipeptide-conjugated nucleosides with the polyA·polyU duplex. The thermal melting curve showed that, in most cases, a slight decrease of T_m was observed, the maximum being 3.8° (*Fig. 1*, *Table*). This means that the existence of dipeptide-

conjugated nucleosides may affect the RNA-duplex stability to some extent. CD Spectra showed that the RNA-typical A-form duplex was maintained in the presence of dipeptide-conjugated nucleosides (*Fig. 2*). The change in peak shape implied a slight change of RNA conformation. Comparing to other cases, a more obvious change was observed in the presence of compound **8a**, which indicated that a stronger interaction existed. The structural difference between **8a** and **13a** resides in the sugar moiety; it is certain that the interactions between the dipeptide-conjugated nucleoside and the RNA duplex are related to the sugar-pucker conformation of the nucleoside, which further affects the base stacking and the nonbonding interactions.

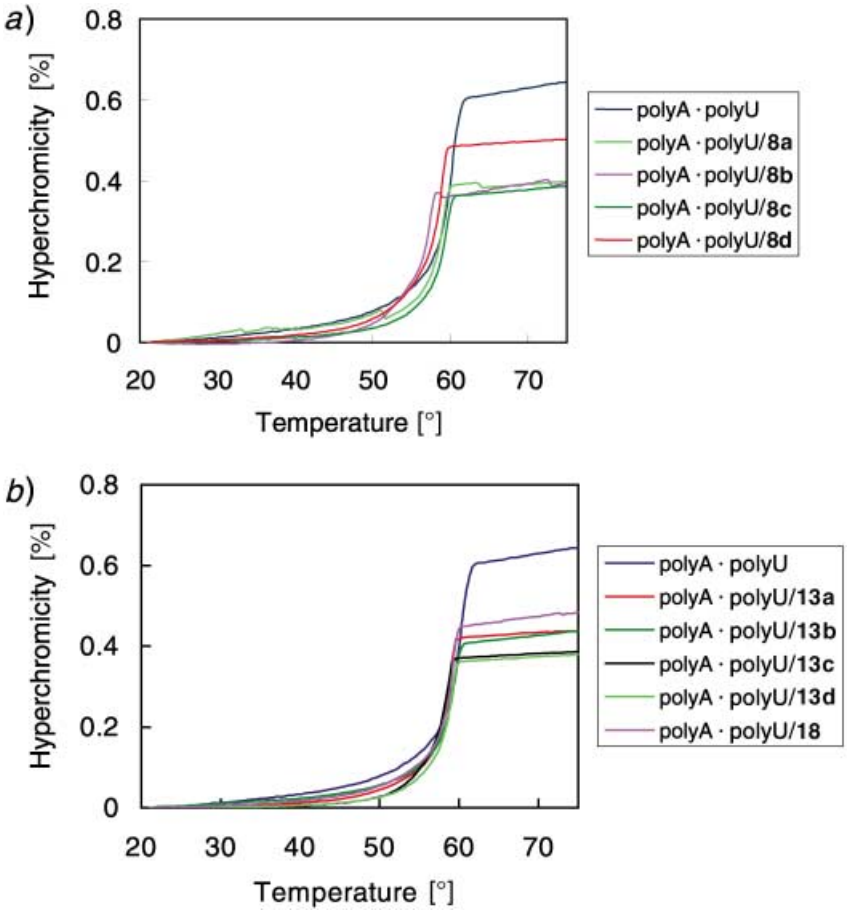


Fig. 1. *Thermal melting curves of polyA·polyU in the presence or absence of the dipeptide-conjugated nucleosides* a) **8a**–**d** *and* b) **13a**–**d** *and* **18**

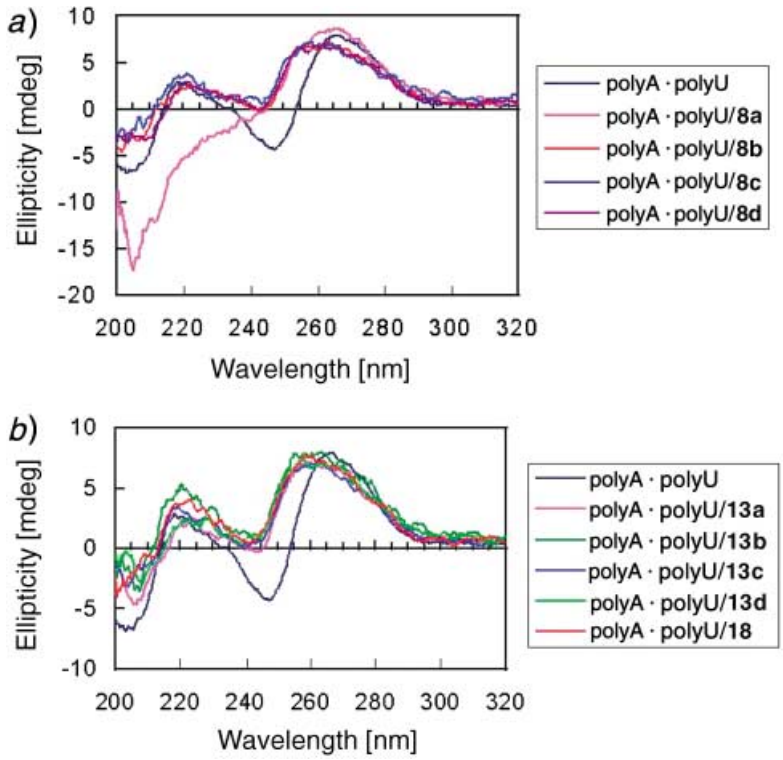


Fig. 2. *CD Spectra of polyA · polyU in the presence or absence of the dipeptide-conjugated nucleosides* a) **8a – d** *and* b) **13a – d** *and* **18**

Table. *Melting Temperatures* T_m *of PolyA · PolyU Duplex in the Presence or Absence of Nucleoside – Peptide Conjugates*

	T_m [°]	ΔT_m [°]
PolyA · polyU/**8a**	59.4	– 0.7
PolyA · polyU/**8b**	56.3	– 3.8
PolyA · polyU/**8c**	60.1	0.0
PolyA · polyU/**8d**	58.0	– 2.1
PolyA · polyU/**13a**	58.0	– 2.1
PolyA · polyU/**13b**	59.5	– 0.6
PolyA · polyU/**13c**	58.2	– 1.9
PolyA · polyU/**13d**	57.2	– 2.9
PolyA · polyU/**18**	59.1	– 1.0
PolyA · polyU	60.1	

This work was supported by the *National Natural Science Foundation of China* (SFCBIC 20320130046).

Experimental Part

1. *General*. All solvents were dried and distilled prior to use. Evaporations were carried out under reduced pressure below 45°. (Aminomethyl)polystyrene (AM) resin was from *Advanced ChemTech*. TLC: GF_{254} silica

gel (*Qingdao Haiyang Chemical Factory*, China). CC = Column chromatography. Optical rotations: *Perkin-Elmer-243-B* polarimeter. NMR Spectra: *Jeol-AL-300* or *Varian-VXR-500* instrument, δ in ppm rel. to $SiMe_4$ as an internal standard, *J* in Hz. ESI-TOF-MS: *ABI Qstar*; in *m/z*.

2. *Dipeptide-Conjugated 3′-Amino-3′-deoxythymidines* **8a**–**d**. *3′-[[(*tert*-Butoxy)carbonyl]amino]-3′-deoxythymidine* (**3**). Di(*tert*-butyl) dicarbonate (2.2 ml, 9.58 mmol) was slowly added to a soln. of **2** (2.0 g, 8.33 mmol) in EtOH (260 ml). The mixture was allowed to react for 1 h at r.t. After evaporation, the residue was purified by CC (silica gel, AcOEt/hexanes 3:2): 2.38 g (83.8%) of **3**. M.p. 115–118°. ^{1}H-NMR ((D_6)DMSO): 11.27 (*s*, NH); 7.74 (*s*, H–C(6)); 6.11 (*t*, H–C(1′)); 5.04 (*s*, OH–C(5′)); 4.05 (*m*, H–C(4′)); 3.64 (*m*, H–C(3′), 2 H–C(5′)); 2.17 (*m*, 2 H–C(2′)); 1.76 (*s*, Me–C(5)); 1.37 (*s*, ^{t}Bu). FAB-MS: 342.1 ($[M+1]^+$).

*3′-[[(*tert*-Butoxy)carbonyl]amino]-3′-deoxythymidine 5′-(Hydrogen Butanedioate)* (**4**). DMAP (788 mg, 6.45 mmol) was added to a soln. of **3** (2.2 g, 6.45 mmol) in pyridine (65 ml). After the solid was dissolved, succinic anhydride (775 mg, 7.74 mmol) was added under Ar, and the mixture was stirred for 30 h at r.t. Then the solvent was evaporated, the residue dissolved in CH_2Cl_2 (80 ml), the soln. washed with 15% aq. citric acid (10 ml) and H_2O (2 × 30 ml) and evaporated, and the residue was purified by CC (silica gel, AcOEt/hexanes 3:2): 2.07 g (73.0%) of **4**. M.p. 107–109°. ^{1}H-NMR ((D_6)DMSO): 7.45 (*s*, H–C(6)); 6.07 (*t*, H–C(1′)); 4.26 (*m*, H–C(3′)); 4.08 (*m*, H–C(4′)); 3.63 (*m*, 2 H–C(5′)); 2.55 (*m*, CH_2CH_2); 2.23 (*m*, 2 H–C(2′)); 1.91 (*s*, Me–C(5)); 1.34 (*s*, ^{t}Bu). FAB-MS: 441.8 ($[M+1]^+$).

*3′-[[(*tert*-Butoxy)carbonyl]amino]-3′-deoxythymidine 5′-(Resin-Bound Butanedioate)* (**6**). To a soln. of 4-nitrophenol (286 mg, 2.06 mmol) and pyridine (0.4 ml) in dioxane (10 ml), **4** (793 mg, 1.80 mmol) and DCC (dicyclohexyl carbodiimide) (793 mg, 3.84 mmol) were added. The mixture was allowed to react for 2 h at r.t., and then the precipitate was filtered off. Aminomethyl (AM) resin (700 mg, 0.91 mmol; 1.3 mmol/g), DMF (6 ml), and DMAP (22 mg; 0.18 mmol) were added to the filtrate. The mixture was stirred for 2 d at r.t., then it was filtered and washed with DMF, MeOH, and Et_2O. After it was dried, Ac_2O (8 ml) and pyridine (20 ml) were added, and the mixture was stirred at r.t. for 1 h to cap the unreacted amino group. Then the mixture was filtered and washed with DMF, MeOH, and Et_2O, and dried.

Dipeptide-Conjugates **8a**–**d** *from 3′-Amino-3′-deoxythymidine 5′-(Resin-Bound Butanedioate)* (**7**). Resin-bound nucleoside **6** was added to 33% CF_3COOH in CH_2Cl_2 and was stirred for 30 min at r.t., then filtered, and washed successively with DMF, MeOH, and Et_2O. The loading amount of nucleoside on resin was determined following the standard procedure. After the resin was dried, standard solid-phase peptide synthesis (Boc strategy) yielded the dipeptide-conjugated nucleosides **8a**–**d**. The final products were purified by CC (silica gel).

L-*Alanyl*-N^1-*[(2*S*,3*S*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*phenylalaninamide* (**8a**): Yield 75.6%. M.p. 162–165°. $[\alpha]_D^{18} = +21.5$ ($c = 0.28$, MeOH). ^{1}H-NMR ((D_6)DMSO)[1]: 7.78 (*s*, H–C(6)); 7.25–7.30 (*m*, 5 arom. H, (Phe)); 6.19 (*t*, H–C(1′)); 3.98–4.32 (*m*, $CH_2(\beta)$(Phe), H–C(α)(Ala)); 3.48–3.75 (*m*, H–C(4′), 2 H–C(5′)); 2.99–3.10 (*m*, H–C(3′)); 2.84 (*m*, H–C(α)(Phe)); 2.04–2.30 (*m*, 2H–C(2′)); 1.79 (*s*, Me–C(5)); 1.23 (*d*, Me(β)(Ala)). ESI-TOF-MS: 460.3002 ($[M+1]^+$; calc. 460.5034).

L-*Alanyl*-N^1-*[(2*S*,3*S*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*alaninamide* (**8b**): Yield 80.4%. M.p. 115.7–116.0°. $[\alpha]_D^{18} = +63.75$ ($c = 0.8$, MeOH). ^{1}H-NMR (CD_3OD)[1]: 7.78 (*s*, H–C(6)); 6.12 (*t*, H–C(1′)); 4.34 (*q*, H–C(α)(Ala^1)); 4.22 (*q*, H–C(α)(Ala^2)); 3.58–3.84 (*m*, H–C(3′), H–C(4′), 2 H–C(5′)); 2.18–2.31 (*m*, 2 H–C(2′)); 1.79 (*s*, Me–C(5)); 1.38 (*d*, Me(β)(Ala^1)); 1.27 (*d*, Me(β)(Ala^2)). ESI-TOF-MS: 384.1197 ($[M+1]^+$; calc. 384.4074).

L-*Alanyl*-N^1-*[(2*S*,3*S*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*leucinamide* (**8c**): Yield 73.5%. M.p. 156–158°. $[\alpha]_D^{18} = +10.17$ ($c = 0.59$, MeOH). ^{1}H-NMR (CD_3OD)[1]: 7.78 (*s*, H–C(6)); 6.12 (*t*, H–C(1′)); 4.22–4.40 (*m*, 2 H–C(2′)); 3.58–3.78 (*m*, H–C(3′), H–C(4′), 2 H–C(5′)); 2.17–2.28 (*m*, $CH_2(\beta)$(Leu)); 1.79 (*s*, Me–C(5)); 1.49–1.63 (*m*, H–C(α)(Ala), H–C(α)(Leu), H–C(γ)(Leu)); 1.26 (*d*, Me(β)(Ala)); 0.89 (*m*, 2 Me(δ)(Leu)). ESI-TOF-MS: 426.1686 ($[M+1]^+$; calc. 426.4871).

L-*Valyl*-N^1-*[(2*S*,3*S*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*phenylalaninamide* (**8d**): Yield 84.3%. M.p. 179–182°. $[\alpha]_D^{18} = +6.23$ ($c = 1.3$, MeOH). ^{1}H-NMR (CD_3OD)[1]: 7.89 (*s*, H–C(6)); 7.22–7.32 (*m*, 5 arom. H(Phe)); 6.21 (*t*, H–C(1′)); 4.46–4.51 (*m*, H–C(4′)); 4.10 (*d*, H–C(α)(Val)); 3.96–4.00 (*t*, H–C(α)(Phe)); 3.82–3.89 (*m*, 2 H–C(5′)); 3.68–3.74

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[1]) The nucleoside numbering is retained.

(*m*, H–C(3′)); 2.89–3.19 (*m*, $CH_2(\beta)$(Phe)); 2.25–2.40 (*m*, 2 H–C(2′)); 1.96–2.08 (*m*, H–C(β)(Val)); 1.88 (*s*, Me–C(5)); 0.94–0.97 (*m*, 2 Me(γ)(Val)). ESI-TOF-MS: 488.1780 ($[M+1]^+$; calc. 488.5565).

3. *Dipeptide-Conjugated 1-(3′-Amino-2′,3′-dideoxy-β-*D*-xylofuranosyl)thymines* **13a–d**. *1-(3′-Azido-2′,3′-dideoxy-5′-*O*-trityl-β-*D*-xylofuranosyl)thymine* (=*1-[3′-Azido-2′,3′-dideoxy-5′-(triphenylmethyl)-β-*D*-threo-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione*; **10**). To a soln. of 5′-*O*-tritylthymidine 3′-(methanesulfonate) (**9**; 3.10 g, 5.52 mmol) in DMF (45 ml), NaN_3 (1.15 g, 17.7 mmol) was added. The mixture was kept at 100° under Ar for 4 h, then it was poured into ice-water (1000 ml). After filtration, the filtrate was purified by CC (silica gel, hexanes/AcOEt 4:1): 1.99 g (71%) of **10**. M.p. 149–151°. ^{1}H-NMR ((D_6)DMSO): 11.35 (*s*, NH); 7.44 (*s*, H–C(6)); 7.35 (*m*, 15 arom. H); 6.07 (*m*, H–C(1′)); 4.56 (*m*, H–C(3′)); 4.27 (*m*, H–C(4′)); 3.18 (*m*, 2 H–C(5′)); 2.73 (*m*, 1 H–C(2′)); 2.06 (*m*, 1 H–C(2′)); 1.66 (*s*, Me–C(5)). FAB-MS: 509.7 ($[M+1]^+$).

*1-(3′-Azido-2′,3′-dideoxy-β-*D*-xylofuranosyl)thymine* (=*1-(3′-Azido-2′,3′-dideoxy-β-*D*-threo-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione*; **11**). A soln. of **10** (2.85 g; 5.60 mmol) in 80% AcOH/H_2O (40 ml) was heated at 120° for 20 min. After evaporation, the residue was purified by CC (silica gel, hexanes/AcOEt 5:4): 1.23 g (83.0%) of **11**. M.p. 154–157°. ^{1}H-NMR ((D_6)DMSO): 11.32 (*s*, NH); 7.48 (*s*, H–C(6)); 6.02 (*m*, H–C(1′)); 5.02 (*m*, OH–C(5′)); 4.45 (*m*, H–C(4′)); 3.98 (*m*, H–C(3′)); 3.68 (*m*, H–C(5′)); 2.72 (*m*, 1 H–C(2′)); 2.07 (*m*, 1 H–C(2′)); 1.78 (*s*, Me–C(5)).

*1-(3′-Amino-2′,3′-dideoxy-β-*D*-xylofuranosyl)thymine* (=*1-(1′-Amino-2′,3′-dideoxy-β-*D*-threo-pentofuranosyl)-5-methylpyrimidine-2,4-(1*H*,3*H*)-dione*; **12**). To a soln. of **11** (2.07 g, 7.78 mmol) in MeOH (80 ml), 10% Pd/C (225 mg) was added. The mixture was hydrogenated under 50 p.s.i. at r.t. for 6.5 h. After filtration, the filtrate was evaporated and the residue extracted repeatedly with AcOEt: 1.61 g (86.2%) of **12**. M.p. 129–131°. ^{1}H-NMR ((D_6)DMSO): 8.17 (*s*, H–C(6)); 6.00 (*q*, H–C(1′)); 3.79 (*m*, H–C(3′), H–C(4′)); 3.67 (*m*, 2 H–C(5′), NH_2–C(3′)); 2.44 (*m*, H–C(2′)); 1.74 (*s*, Me–C(5)). FAB-MS: 241.0 ($[M+1]^+$).

Dipeptide Conjugates **13a–d** *from* **12**. As described for **8a–d**.

L-*Alanyl*-N^1-*[(2*S*,3*R*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*phenylalaninamide* (**13a**): Yield 82.9%. M.p. 150–153°. $[\alpha]_D^{18} = +51.67$ ($c = 0.31$, MeOH). ^{1}H-NMR ((D_6)DMSO)[1]): 7.78 (*s*, H–C(6)); 7.24–7.33 (*m*, 5 arom. H (Phe)); 5.96 (*t*, H–C(1′)); 4.28–4.52 (*m*, 2 H–C(2′)); 3.96–4.01 (*m*, H–C(4′)); 3.86–3.91 (*m*, H–C(3′)); 3.49–3.59 (*m*, 2 H–C(5′)); 2.79–3.09 (*m*, $CH_2(\beta)$(Phe)); 2.44–2.53 (*m*, H–C(α)(Phe)); 1.86–1.92 (*q*, H–C(α)(Ala)); 1.86 (*s*, Me–C(5)); 1.20 (*d*, Me(β)(Ala)). ESI-TOF-MS: 460.3002 ($[M+1]^+$; calc. 460.5034).

L-*Phenylalanyl*-N^1-*[(2*S*,3*R*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*phenylalaninamide* (**13b**): Yield 93.2%. M.p. 149–150°. $[\alpha]_D^{18} = +46.75$ ($c = 0.40$, MeOH). ^{1}H-NMR (CD_3OD[1]): 7.70 (*s*, H–C(6)); 7.19–7.37 (*m*, 10 arom. H (Phe^1,Phe^2)); 5.84 (*t*, H–C(1′)); 4.46–4.56 (*m*, H–C(2′)); 4.03–4.10 (*m*, H–C(3′), H–C(4′)); 3.71 (*m*, 2 H–C(5′)); 4.10 (*d*, H–C(α)(Phe^2)); 2.95–3.31 (*m*, 4 H, $CH_2(\beta)$(Phe^1,Phe^2)); 2.43–2.52 (*m*, H–C(α)(Phe^1)); 1.88 (*s*, Me–C(5)); 1.21–1.28 (*m*, H–C(α)(Phe^2)). ESI-TOF-MS: 536.1493 ($[M+1]^+$; calc. 536.5993).

L-*Alanyl*-N^1-*[(2*S*,3*R*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*valinamide* (**13c**): Yield 79.8%. M.p. 167–168°. $[\alpha]_D^{18} = +39.0$ ($c = 0.30$, MeOH). ^{1}H-NMR (CD_3OD[1]): 7.76 (*s*, H–C(6)); 5.89 (*t*, H–C(1′)); 4.17–4.53 (*m*, 2 H–C(2′)); 3.96–4.03 (*m*, H–C(3′), H–C(4′)); 3.60–3.74 (*m*, 2 H–C(5′)); 3.51 (*d*, H–C(α)(Val)); 1.99–2.14 (*m*, H–C(α)(Ala), H–C(β)(Val)); 1.80 (*s*, Me–C(5)); 1.26 (*d*, Me(β)(Ala)); 0.92–0.99 (*m*, 2 Me(γ)(Val)). ESI-TOF-MS: 412.1451 ($[M+1]^+$; calc. 412.4606).

L-*Phenylalanyl*-N^1-*[(2*S*,3*R*,5*R*)-tetrahydro-2-(hydroxymethyl)-5-(1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*leucinamide* (**13d**): Yield 81.8%. M.p. 147–149°. $[\alpha]_D^{18} = +24.47$ ($c = 0.38$, MeOH). ^{1}H-NMR (CD_3OD[1]): 7.62 (*s*, H–C(6)); 5.74 (*t*, H–C(1′)); 4.37–4.45 (*m*, 2 H–C(2′)); 3.93–3.98 (*m*, H–C(4′)); 3.52–3.67 (*m*, H–C(3′), 2 H–C(5′)); 2.87–2.99 (*m*, $CH_2(\beta)$(Phe)); 2.34–2.44 (*m*, H–C(α)(Phe)); 1.78 (*s*, Me–C(5)); 1.43–1.70 (*m*, H–C(α)(Leu), H–C(γ)(Leu), $CH_2(\beta)$(Leu)); 0.76–0.89 (*m*, 2 Me(δ)(Leu)). ESI-TOF-MS: 502.1980 ($[M+1]^+$; calc. 502.5831).

4. *Dipeptide Conjugate* **18**. *2′-[[(*tert*-Butoxy)carbonyl]amino]-2′-deoxyuridine* (**15**). To a suspension of **14** (778 mg, 3.20 mmol) in MeCN (200 ml), (*tert*-butyl) dicarbonate (0.8 ml, 3.48 mmol) and Et_3N (0.7 ml, 5.02 mmol) were added. The mixture was stirred overnight at r.t. and then evaporated. The residue was purified by CC (silica gel, AcOEt/hexanes 5:2): 760 mg (69.2%) of **15**. M.p. 224–225°. ^{1}H-NMR ((D_6)DMSO): 11.44 (*s*, H–N(3)); 7.85 (*s*, H–C(6)); 5.85 (*d*, H–C(5)); 5.56 (*d*, H–C(1′)); 5.16 (*m*, 1 H–C(2′)); 4.18–3.99 (*m*, H–C(3′), H–C(4′)); 3.57 (*m*, 2 H–C(5′)); 1.33 (*s*, ^{t}Bu). FAB-MS: 343.9 ($[M+1]^+$).

L-*Phenylalanyl*-N^1-*[(2*R*,3*R*,4*S*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)-2-(1,2,3,4-tetrahydro-2,4-dioxopyrimidin-1-yl)furan-3-yl]*-L-*phenylalaninamide* (**18**). As described for **8a–d**, except that the intermediates **16a**/**16b** and **17a**/**17b** were used directly without separation. After the conjugate was released from the resin, it

was purified by CC (silica gel): **18** (91.6%). $[\alpha]_D^{18} = +21.3$ ($c = 0.27$, MeOH). M.p. 156–158°. ^{1}H-NMR (CD_3OD)[1]); 8.02 (d, $J(5,6) = 8.4$, H–C(6)); 7.36–7.17 (m, 10 arom. H (Phe^1,Phe^2)); 6.02 (t, H–C(1′)); 4.68 (m, H–C(α)(Phe)); 4.55 (m, H–C(α)(Phe)); 4.21 (m, H–C(2′)); 4.07 (m, H–C(3′)); 3.91 (m, H–C(4′)); 3.75 (m, H–C(5′)); 3.17 (m, CH_2(β)(Phe^1)); 2.86 (m, CH_2(β)(Phe^2)). ESI-TOF-MS: 538.1515 ($[M+1]^+$; calc. 538.5721).

5. *Circular-Dichroism and Thermal-Melting Measurements.* CD Spectra: *Jasco J-715* spectropolarimeter; at 15° in thermostatically controlled 1-cm cuvette. Thermal denaturation studies of polyA/polyU: *Varian Cary 300*. CD Spectra and T_m values in the absence and presence of synthetic dipeptide-conjugated nucleosides were determined in buffer soln. containing 10 mM Na_2HPO_4, 0.14M NaCl, and 1.0 mM EDTA (pH 7.2). The soln. containing dipeptide-conjugated nucleoside was mixed with equimolar amounts of polyA and polyU per nucleoside. The concentration of dipeptide-conjugated nucleosides was 40 and 60 μM in the case of thermal-melting measurements and CD spectra, respectively.

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Received June 2, 2003