DOI: 10.1002/ejoc.201301781

Tailoring Peptide–Nucleotide Conjugates (PNCs) for Nucleotide Delivery in Bacterial Cells

Swarup De,[a] Elisabetta Groaz,[a] and Piet Herdewijn*[a]

Keywords: Bioorganic chemistry / Peptides / Nucleotides / Peptide–nucleotide conjugates

The design and synthesis of peptide-2′-deoxythymidine-5′-*O*-monophosphate conjugates as potential active delivery systems for nucleotides into auxotrophic *E. coli* strains is presented. A series of oligopeptides were allowed to react with 5′-*O*-(dibenzylphosphate)-2′-deoxythymidine or its suitably 3′-derivatized analogues to give the relevant peptide–nucleotide adducts, by the formation of a biolabile chemical connection. Using strategies based on the principles of orthogonal protection and activation, rational variations were made to the linker and the peptide moiety in order to tune the metabolic stability of the conjugates.

Introduction

The ability to encode uncharted heritable information in polymers other than DNA and RNA relies upon chemical diversification and enzymatic proliferation of artificial nucleic acids (XNAs).[1,2] With the aim of controlling and preventing cross-contamination, new synthetic genetic sequences will have to be conceived to satisfy a principle of orthogonality with respect to wild biodiversity, leading to the establishment of self-reliant genetic enclaves.[3] Fundamental progress in enzyme evolution has recently been made with the discovery of engineered polymerase mutants that are able to recognize and efficiently incorporate backbone-modified nucleotide building blocks [hexitol (HNA), cyclohexene (CeNA), arabino (ANA), 2′-deoxy-2′-fluoroarabino (FANA), threose (TNA), and locked (LNA) nucleic acids] from a DNA template. XNA sequences up to 72 nt (nucleotides) in length were generated following canonical base-pairing selectivity, and reverse-transcribed into DNA.[4] Nonetheless, major challenges remain if XNAs are to reach their true evolutionary potential as genetic reprogramming tools in vivo.[5] The molecular assembly of xenopolymers within the host cell requires the intracellular availability of preactivated unnatural precursors, since most modified nucleoside substrates are poorly phosphorylated by host kinases. However, negatively charged nucleotides suffer from low cell-penetrating ability and a known instability as a result of their propensity to undergo hydrolysis to the phosphate-free state of nucleosides by exocytoplasmic phosphatases before being taken up.

[a] Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven,
Minderbroedersstraat 10, 3000 Leuven, Belgium
E-mail: piet.herdewijn@rega.kuleuven.be
http://medchem.rega.kuleuven.be/

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201301781.

Given these facts, we envisaged that specifically tailored nucleotide conjugates might act as active delivery systems, exploiting membrane uptake systems naturally occurring in bacterial cells for the internalization of essential nutrients, to eventually facilitate nucleotide delivery across the cytoplasmic membrane. In particular, the rationale for our study hinges on the significance of peptide transporters in prokaryotic microorganisms, which are best represented by the vast family of binding-protein-dependent permeases.[6] The derivatization of ligands with peptides is an attractive concept in medicinal chemistry, as it can give cellular access to synthetic antimicrobial compounds that might be difficult or impossible to be delivered by direct means.[7,8] Microbial permeases are multicomponent ATP (adenosine triphosphate) binding cassette (ABC) proteins that mediate the active transport of peptides, which act as a source of amino acids, carbon, nitrogen, and energy.[9,10] In Gram-negative species, three classes of peptide permeases (Dpp, Tpp, and Opp) have been found, which have complementary selectivities for different conformations, sizes, and compositions of oligopeptide sequences, thus ensuring complete uptake from the peptide pool. Of the periplasmic proteins required for initial molecular recognition and binding by permeases, the oligopeptide binding protein OppA is the most abundant (7–10%) and structurally well-defined.[11,12] In vitro binding studies on peptide complexes of *E. coli* OppA highlighted high affinity and broad substrate specificity for small peptides comprising two up to five or six L-amino acids, regardless of their sequence.[13,14] Further investigations have suggested a preference for peptides containing Ala, Gly, Phe,[13] and basic residues,[15] in particular lysines, which may result from a negatively charged surface in the vicinity of the initial binding site.

It has been suggested that the presence of free α-amino and carboxyl groups at the N- and C-terminals, respectively, were necessary for binding to *E. coli* OppA and subsequent

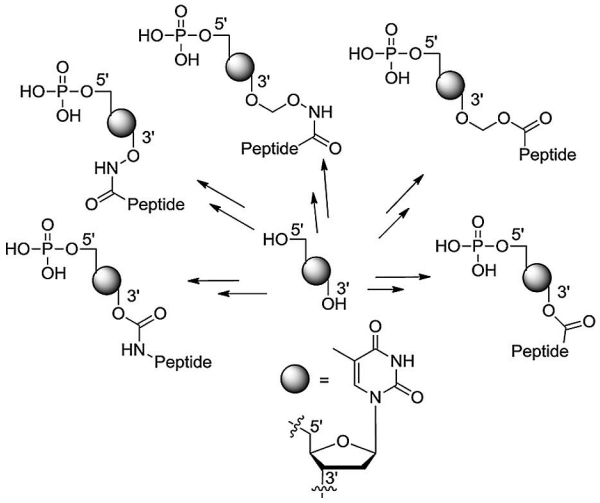
transmembrane translocation into the cell to take place, but protected peptides were also transported, albeit less effectively.[13] However, the presence of a free amino group might trigger extracellular metabolism of the peptide conjugate by peptidases or degradation by chemical means, owing to its high reactivity.

With this knowledge in mind, an approach in which oligopeptides are conjugated to nucleotides by a bioreversible linkage, appeared to be logical in an attempt to develop a new cellular uptake system. It is, however, anticipated that the success of this proposal would strongly depend on the metabolic stability of the relevant adducts, which should be sufficiently stable to be able to cross the cell wall without undergoing premature degradation, but still be cleaved at the linker site once in the presence of intracellular enzymes. To assess the viability of our model, we selected 5′-thymidylate (dTMP) nucleotide conjugates as initial synthetic targets, for which reliable nutritional selections using *E. coli* mutants lacking thymidylate synthase are well established. A conjugate derived from a pyridoxal-pyroglutamyl-tripeptido-nucleotide, which could release the dTMP synthon by an autocatalytic elimination process, was prepared, but purification and scaling-up of the synthesis of this adduct was not straightforward.[16] In this paper, we describe a further elaboration of this concept with the synthesis of a diverse range of peptide–nucleotide conjugate (PNCs) analogues, prepared by covalently linking di- to pentapeptidic linear chains, through various spacers, to the 3′-position of the ribose moiety. Amino acidic residues and chemical connections between dTMP and peptides were systematically examined, as summarized in Figure 1. The α-amino group at the *N*-terminus was either unmodified or protected as a formylamide (For), which can be hydrolytically cleaved by *E. coli* peptide deformylase. This enzyme is known to show a preference for substrates containing *N*-formyl methionine, followed by *N*-formyl glycine.[17] The α- or β-carboxyl groups present in the second or third residue, together with the amino functionality in side-chain of Lys, were utilized to form the connecting bond. Following internalization of the conjugate in the cell, the peptide might be hydrolysed by *E. coli* PepN, the principal cytosolic aminopeptidase enzyme responsible for the hydrolysis of peptides, whose activity varies in the following order: Arg > Ala > Lys > Gly.[18]

Results and Discussion

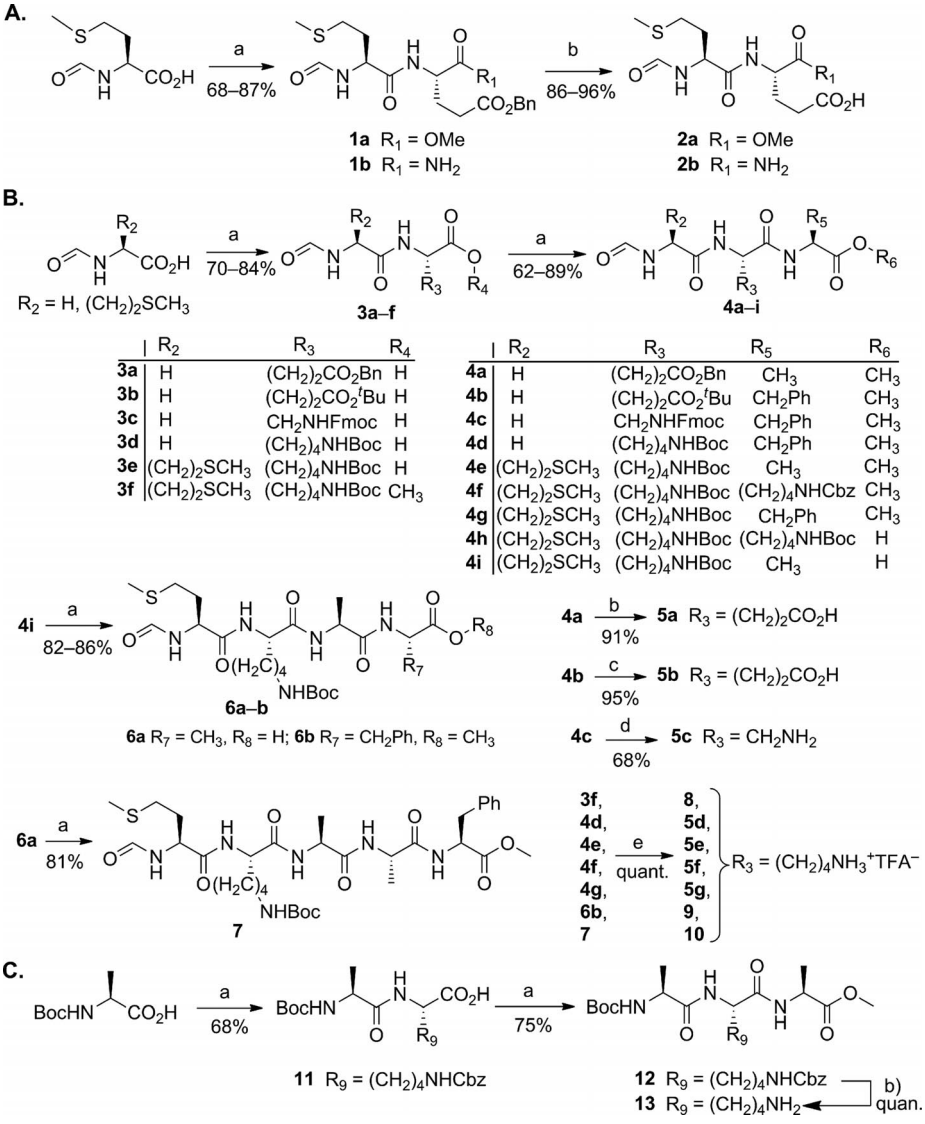
Several strategies have been described in the literature to prepare peptide–oligonucleotide conjugates connected by variously functionalized linkers, such as a thioether, a disulfide, an amide, and an oxime moiety etc.[19] For our study, we selected five different linkers (Figure 1), which were assumed to be liable to cleavage inside the bacterial cell, either by a chemical or an enzymatic mechanism, i.e., carbamate (OCONH), ester (OCO), oxyamide (ONHCO), oxymethyleneoxyamide (OCH_2ONHCO), and oxymethyleneoxy ester (OCH_2OCO). The corresponding peptide–nucleotide conjugates (PNCs) were synthesized by the reaction of the 3′-hydroxy group of dTMP with the side-chain functionalities of an oligopeptide, for example an amino or a carboxylic acid group.

Firstly, di-, tri-, tetra-, and pentapeptides were conveniently prepared according to the NHS (*N*-hydroxysuccinimide) activated ester method[20] starting from commercially available *N*-Boc (*tert*-butoxycarbonyl) protected/formylated amino acids, as shown in Scheme 1. In the first step, NHS-activated carboxy ester derivatives were formed and subsequently coupled with an incoming amino acid without the need for protection at the C-terminal. The side-chain functionalities were orthogonally protected as Boc, Fmoc (fluorenylmethyloxycarbonyl), Cbz (benzyloxycarbonyl), OBn, or *t*Bu during peptide synthesis, and the protecting groups were removed prior to coupling to the free



Linker	Peptide
OCONH	For-M-K-OMe, For-G-K-F-OMe, For-G-βA-F-OMe, For-M-K-F-OMe, For-M-K-A-OMe, For-M-K-K-OMe, For-M-K-A-F-OMe, For-M-K-A-A-F-OMe, A-K-A-OMe, A-K-A
ONHCO	For-M-E-OMe
OCH_2ONHCO	For-M-E-OMe, For-M-E-NH_2
OCH_2OCO	For-G-E-A-OMe
OCO	For-M-K-K, For-G-E-F

Figure 1. Chemical diversity in the design of novel peptide-nucleotide conjugates (PNCs) as delivery system of natural and modified nucleotides in prokaryotic cells.

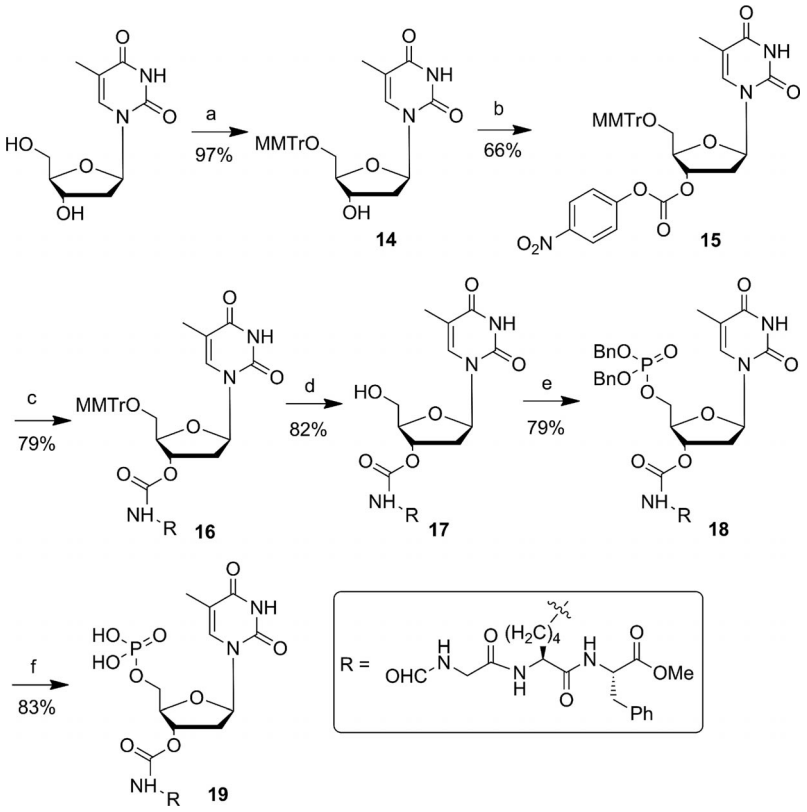


Scheme 1. Preparation of dipeptides **2a**, **2b**, and **8**, tripeptides **4h**, **5a**–**5g**, and **13**, tetrapeptide **9**, and pentapeptide **10**. Reagents and conditions: (a) (i) NHS, DCC (*N,N′*-dicyclohexylcarbodiimide), THF, 0 °C to room temp., 2–12 h, (ii) amino acid, $NaHCO_3$, THF/H_2O, 0 °C to room temp., 16 h; (b) Pd/C (10%; Degussa), H_2, EtOH or MeOH, room temp., 6–24 h; (c) TFA, CH_2Cl_2, 0 °C to room temp., 2 h; (d) Et_3N/CH_2Cl_2 (2:1), room temp., 72 h; (e) TFA, thioanisole, CH_2Cl_2, room temp., 4 h.

3′-OH group of thymidine. For the acidic deprotection of the *N*-Boc group, thioanisole was used as a radical scavenger;[21] an excess of Pd/C was used in order to avoid catalyst poisoning during removal of the benzyl group of methionine-containing peptides. In the case of peptide **4c**, the Fmoc group was removed using Et_3N in dichloromethane. Ethanol was used as solvent[22] for the *N*-Cbz deprotection of **12** to give **13**, since in the presence of methanol a *N*-methylated by-product was formed instead.

The construction of PNCs can be accomplished either by linking the peptide at the 3′-position of thymidine followed by 5′-*O*-phosphorylation, or by reversing the order of this reaction sequence. For the synthesis of our first PNC analogue **19**, featuring a carbamoyl linker (OCONH), it was decided to use the first of these two approaches, as shown in Scheme 2. In the first step, the 5′-hydroxy group of 2′-deoxythymidine was selectively protected with a MMTr (4-monomethoxytrityl) group[23] to give 5′-*O*-MMTr-2′-deoxythymidine **14**. This compound was then activated at its 3′-OH group using carbonyldiimidazole (CDI)[24,25] or 4-nitrophenyl chloroformate[26] (via intermediate **15**), and subsequent reaction with the ε-amino group of the lysine residue of peptide **5d** gave compound **16**. After detritylation, the phosphate group was introduced at the 5′-position using dibenzyl-*N*,*N*-diisopropyl phosphoramidite and hydrogen peroxide as oxidizing agent. The resulting thymidine-5′ dibenzyl phosphate product (i.e., **18**) was subjected to hydrogenation in the presence of Pd/C to produce the free phosphate functionality.

However, this strategy could not be used for the synthesis of *N*-formyl-L-methionine-containing analogues, presumably due to the instability of the thiomethyl group during



Scheme 2. Synthesis of 3′-*O*-carbamoyl peptide conjugate of dTMP **19**. Reagents and conditions: (a) MMTrCl, Et_3N, DMAP (4-dimethylaminopyridine), DMF, room temp., 4 h; (b) 4-nitrophenyl chloroformate, Py, CH_2Cl_2, 0 °C to room temp., 24 h; (c) peptide **5d**, Et_3N, CH_2Cl_2, room temp.; (d) AcOH (80%), room temp., 3.5 h; (e) (i) dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole (0.45 M in MeCN), CH_2Cl_2, room temp., 12 h, (ii) H_2O_2, –40 °C to room temp., 2 h; (f) Pd/C (10%; Degussa), H_2, Et_3N, MeOH.

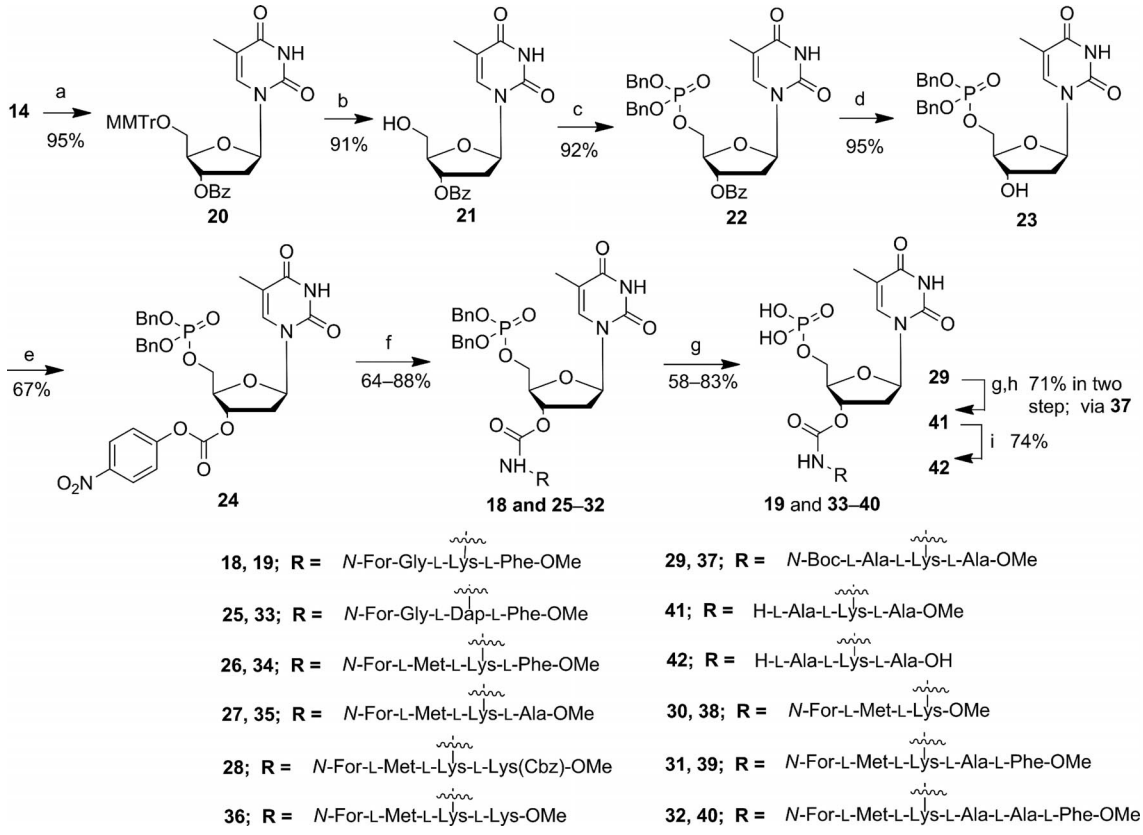
the oxidation of P^{III} to P^{V}, even when milder oxidizing reagents, such as a dilute solution of I_2 in pyridine/THF/H_2O, were used.[27,28] Therefore, the route to compound **19** was repeated using 3′-benzoyl thymidine **21** as starting material, as shown in Scheme 3.

The dibenzylphosphate group at the 5′-position of **21** was introduced first, followed by peptide conjugation. The same synthetic method was also used to give nine further PNCs, **18** and **25**–**32**, built on a carbamoyl linker.

With regard to this second route, it was necessary to protect the secondary OH group to obtain phosphorylated nucleoside **23** in good yield.[29–31] Thus, we elected to protect the free hydroxy functionality of compound **14** using benzoyl chloride.[32] Standard MMTr deprotection proceeded as expected using AcOH (80%) to give 3′-*O*-benzoyl-2′-deoxythymidine **21**, which was in turn phosphorylated with dibenzyl *N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole[33] at the 5′-position. Subsequent oxidation in the presence of hydrogen peroxide gave 5′-dibenzylphosphate-3′-*O*-benzoyl-2′-deoxythymidine **22** in 92% yield over two steps. Debenzoylation was effected by treatment of **22** with saturated methanolic ammonia to give key intermediate **23** in 95% yield. This compound served as a common substrate for the subsequent coupling step to explore various 3′-*O* linkers. As shown in Scheme 3, the 3′-OH group was derivatized with 4-nitrophenyl chloroformate in pyridine to give 4-nitrophenyl carbonate **24** in moderate yield.[34] The activated carbonate was then treated with amino peptides **5c**–**5g**, **8**–**10**, and **13** to generate carbamates **18** and **25**–**32** in yields ranging from 64 to 88%, depending on the peptide used. Finally, hydrogenolysis of the benzyl groups was carried out under mild conditions, in order to prevent reduction of the double bond of the thymine ring, using palladium on carbon (10%; Degussa) or $Pd(OH)_2$/C (20%) at atmospheric pressure, smoothly giving phosphate derivatives **19** and **33**–**40** in 58–83% yield.

Notably, debenzylation by hydrogenation was successful for conjugates containing methionine in the peptide chain. A larger excess of Pd/C was used during these reactions to avoid poisoning of the catalyst by the thiomethyl group. In most cases, except for compound **36** where a free amino group is present in the peptidic side-chain, 2 equiv. of Et_3N was used to neutralize the strong acidity of the phosphoric acid group during di-*O*-benzyl deprotection. The terminal amino group of **37** was deprotected with TFA (trifluoroacetic acid) to give conjugate **41**, which was then treated with LiOH to liberate the terminal carboxyl group and produce compound **42**.

The synthesis of 3′-oxyamide-linked PNC **48** is shown in Scheme 4. 3′-Oxyamine intermediate **46** was synthesized



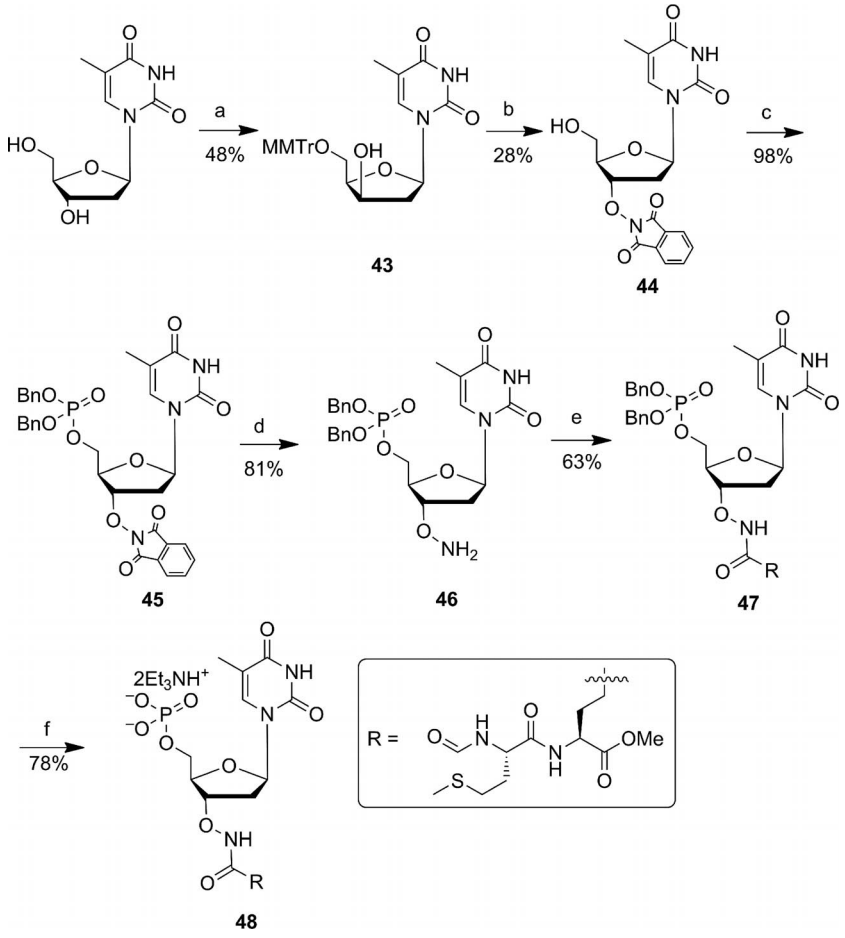
Scheme 3. Synthesis of 3′-carbamoyl peptide conjugates of dTMP **19** and **33**–**42**. Reagents and conditions: (a) BzCl, Py, 0 °C, 3 h; (b) AcOH (80%), room temp., 3.5 h; (c) (i) dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole (0.45 M in MeCN), CH_2Cl_2, room temp., 12 h, (ii) H_2O_2, –40 °C to room temp., 2 h; (d) NH_3 (7 N in MeOH), 0 °C to room temp., 24 h; (e) 4-nitrophenyl chloroformate, Py, CH_2Cl_2, 0 °C to room temp., 24 h; (f) peptide-NH_2 (**5c**–**5g**, **8**–**10** or **13**), Et_3N, CH_2Cl_2, room temp.; (g) Pd/C (10%; Degussa), H_2, MeOH or EtOH, room temp., 24 h; (h) TFA, thioanisole, H_2O, room temp., 3 h; (i) LiOH, THF/MeOH/H_2O (1:1:1), room temp., 3 h.

starting from 2′-deoxythymidine according to modified literature procedures.[35–37] 5′-*O*-MMTr-2′-deoxy-*xylo*-thymidine **43** was obtained from 2′-deoxythymidine in 48% yield over three steps.[38]

The protected hydroxylamine functionality was formed by Mitsunobu inversion using *N*-hydroxyphthalimide. Removal of the MMTr group and phosphorylation at the 5′-position, as described above, yielded protected hydroxylamine **45**, which was then treated with methylamine (4%) to restore the 3′-ONH_2 group. Although stronger nucleophilic reagents like hydrazine hydrate or hydroxylamine are more efficient for phthalimide deprotection, our reagent of choice was diluted methylamine, to minimize the rate of side-reactions at the protected 5′-phosphate moiety.

Coupling between the 3′-oxyamino group of compound **46** and the free carboxylic acid group of dipeptide **2a** in the presence of DCC led to benzyl-protected oxyamide **47**, which was subjected to catalytic hydrogenation using Pd/C and Et_3N at atmospheric pressure. Final purification by RP (reverse-phase) HPLC yielded **48** as a triethylammonium salt. The common key intermediate in the synthesis of 3′-oxymethyleneoxyamide PNCs **57** and **58** is compound **54** (Scheme 5), which can be coupled with different peptides with a free carboxylic acid group in their side-chains.[39,40] Compound **54** was obtained from 5′-*O*-protected-3′-*O*-methylenethiomethyl-2′-deoxythymidine **50**, which was synthesized by a modified literature procedure.[41] The acid-stable TBDPS (*tert*-butyldiphenylsilyl) group was selected to mask the 5′-position in view of potential complications at a later stage of the synthetic scheme. The 3′-thioacetal group was converted by treatment with sulfuryl chloride into the corresponding chloromethyl ether. Without further purification, this compound was treated with *N*-hydroxyphthalimide to give 3′-*O*-aminooxymethylene derivative **51**. The 5′-TBDPS protection was removed by treatment with $Et_3N{\cdot}3HF$, and the free 5′-hydroxy group was phosphorylated using our general protocol. Phthalimide deprotection followed by DCC coupling with dipeptides **2a** and **2b** yielded conjugates **55** and **56**, respectively. Benzyl deprotection and HPLC purification as described above, yielded the 5′-*O*-monophosphates of the 2′-deoxythymidine-3′-oxymethyleneoxyamide peptide conjugates as triethylammonium salts, i.e., **57** and **58**.

Due to its propensity to undergo cleavage by cellular carboxyesterase, the oxymethylene ester moiety has been described in the literature in the context of bioavailable antiretroviral prodrugs based on acyclic nucleosides,[42,43] and also as a biolabile 2′-*O*-protecting group for the devel-



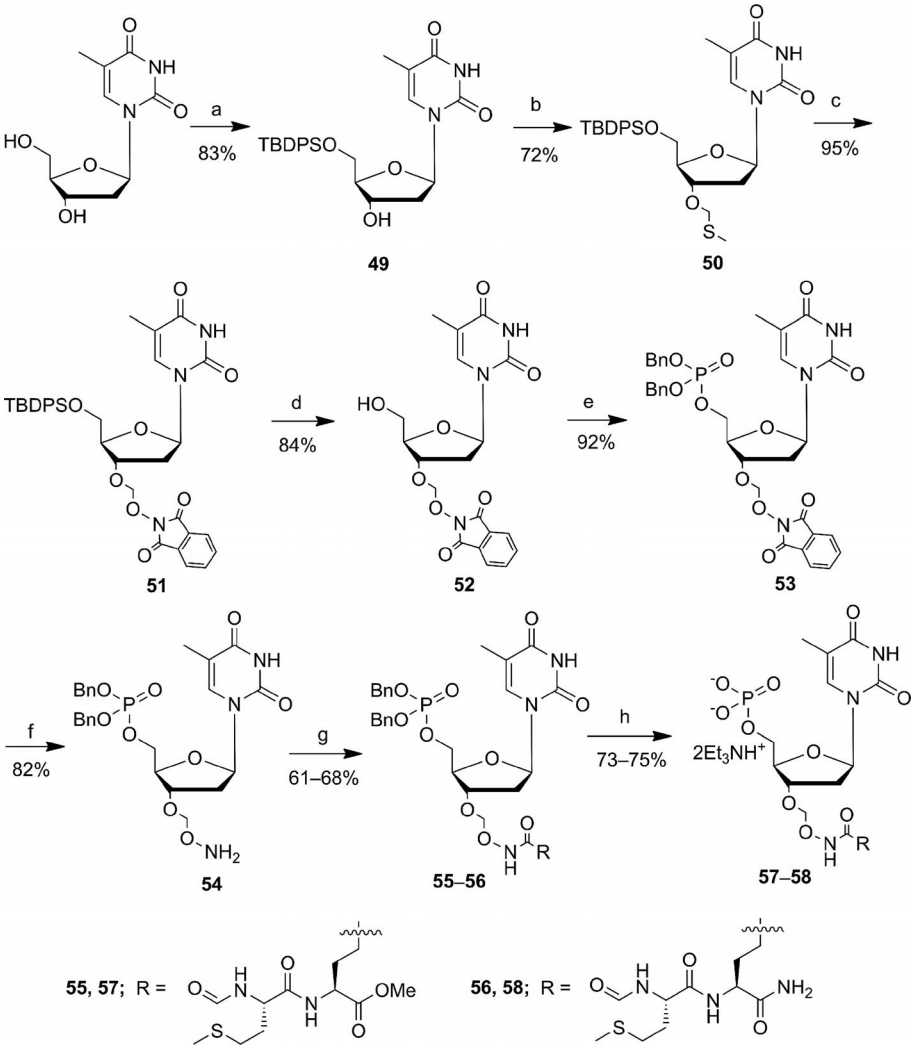
Scheme 4. Synthesis of the 3′-oxyamide peptide conjugate of dTMP **48**. Reagents and conditions: (a) (i) MMTrCl, Et_3N, Py, room temp., 16 h, (ii) MsCl, Et_3N, room temp., 2 h, (iii) NaOH, EtOH, reflux, 1.5 h; (b) (i) *N*-hydroxy phthalimide, PPh_3, DIAD, toluene, 0 °C to room temp., 2 h, (ii) AcOH (80%), room temp., 3 h; (c) (i) dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole (0.45 M in MeCN), CH_2Cl_2, room temp., 12 h, (ii) H_2O_2, –40 °C to room temp., 2 h; (d) $MeNH_2$ (4%), EtOH, room temp., 0.5 h; (e) dipeptide **2a**, DCC, DMAP, CH_2Cl_2/DMF, room temp., 24 h; (f) Pd/C (10%; Degussa), H_2, MeOH, Et_3N, room temp., 24 h.

opment of short synthetic double-stranded RNA sequences with therapeutic applications in vivo.[44–46] The formation of 3′-*O*-methylacyloxy PNC **62** essentially followed known procedures for similar transformations; previously synthesized intermediate **50** was activated, and the β-carboxyl group of tripeptide **5a** was used as a nucleophile (Scheme 6).

A first attempt using NIS (*N*-iodosuccinimide)/NCS (*N*-chlorosuccinimide) as the activating agent[47] did not give satisfactory yields of compound **59**. Therefore, we switched to the sulfuryl chloride method, which was more successful.[48,49] TBDPS deprotection using $Et_3N{\cdot}3HF$ and phosphorylation at the 5′-position was carried out as shown in Scheme 5 to give compound **61**. Finally, $Pd(OH)_2$-mediated hydrogenolysis in the presence of Et_3N gave triethylammonium salt **62** in moderate yield, due to undesirable side-reactions involving cleavage of the linker, giving rise to dTMP and the peptide as by-products.

The synthesis of PNCs with an ester linker was thought to be particularly promising in view of the known susceptibility of this functional group to intracellular hydrolysis by bacterial esterases. Analogue **65** was obtained from previously prepared protected 2′-deoxythymidine-5′-*O*-monophosphate **23** by standard coupling to the free terminal carboxylate of tripeptide **4h** in the presence of DCC and DMAP as catalyst,[50] in good yield. Deprotection of the dibenzyl phosphate by catalytic hydrogenation led to **64**, which, without further purification, underwent removal of the NH-Boc protecting groups in the presence of TFA to give final compound **65** as a TFA salt in 62% yield over two steps (Scheme 7). When Cbz was used in place of Boc, attempts to remove all the protecting groups in one step by catalytic hydrogenation resulted in a complex mixture of degradation products.

Key intermediate 5′-*O*-(dibenzylphosphate)-2′-deoxythymidine **23** could also be prepared by an alternative sequence of protection/deprotection steps. The synthesis, as shown in Scheme 8, started with the bis-silylation of thymidine to form intermediate **66**, which was selectively deprotected at the 5′-position in good yield (59%) using a mixture of TFA and H_2O (10:1).[51] Compound **67** was then subjected to standard phosphorylation followed by



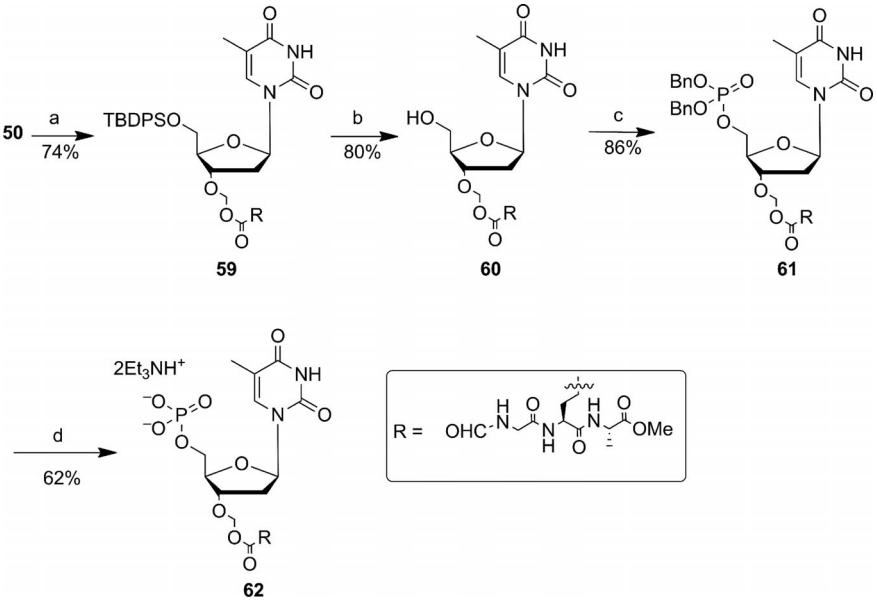
Scheme 5. Synthesis of 3′-oxymethyleneoxyamide peptide conjugates of dTMP **57** and **58**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, room temp., 4 h; (b) DMSO, Ac_2O, AcOH, room temp., 48 h; (c) (i) SO_2Cl_2, CH_2Cl_2, 0 °C to room temp., 2 h, (ii) *N*-hydroxyphthalimide, DBU (1,8-diazabicycloundec-7-ene), CH_2Cl_2, room temp., 24 h; (d) $Et_3NH{\cdot}3HF$, THF, room temp., 36 h; (e) (i) dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole (0.45 M in MeCN), CH_2Cl_2, room temp., 12 h, (ii) H_2O_2, –40 °C to room temp., 2 h; (f) $MeNH_2$ (4%), EtOH, room temp., 0.5 h; (g) dipeptide **2a** or **2b**, DCC, DMAP, CH_2Cl_2/DMF, room temp., 24 h; (h) Pd/C (10%; Degussa), H_2, Et_3N, MeOH, room temp., 24 h.

TBDMS (*tert*-butyldimethylsilyl) cleavage to give the required substrate (i.e., **23**) with which to carry out peptide coupling. In this step, the use of the milder reagent $Et_3N{\cdot}HF$ was found to give higher yields than TBAF (tetrabutylammonium fluoride). In the presence of the free β-carboxyl group of tripeptide **5b**, DCC, and DMAP, **23** was cleanly converted into conjugate **69** and eventually into its triethylammonium salt **70**.

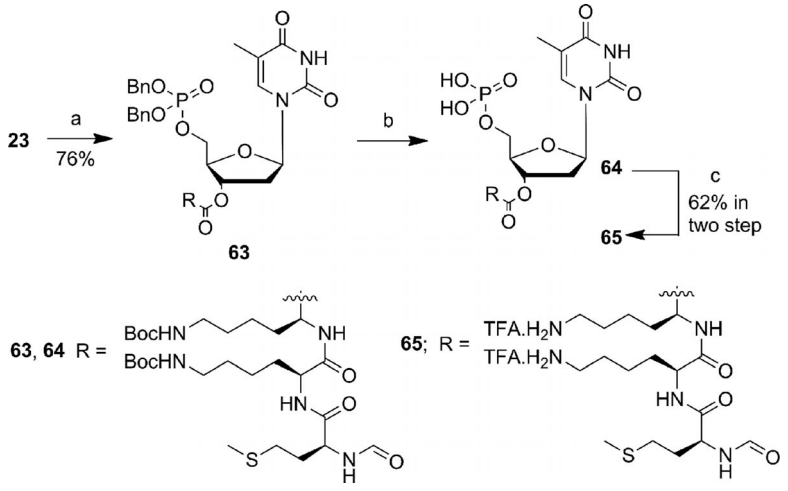
Conclusions

The derivatization of ligands with peptides can be used to achieve a large improvement in the cellular uptake of hydrophilic structures. We have described the application of this methodology to nucleotides, which has led to the development of a wide variety of peptide-5′-thymidylate conjugates featuring linker moieties such as carbamate, ester, oxyamide, oxymethyleneoxyamide, and oxymethyleneoxy ester, all of which are potentially susceptible to enzymatic or chemical cleavage inside the bacterial cell. Final deprotection of all structures was achieved in high yield by palladium-catalysed hydrogenolysis. Of particular note is the unexpected tolerance of the thiomethyl functionality present in methionine-containing PNCs of the debenzylation conditions.

Biological studies on the metabolic stability and functional activity of all of the synthesized constructs in culture medium and bacterial cells (ThyA$^-$ auxotrophic *E. coli* mutants) are currently being performed, and the results will be reported in due course. With a view to the development of a xenonucleotide delivery system in bacterial cells, the introduction of other types of residues is also being explored.



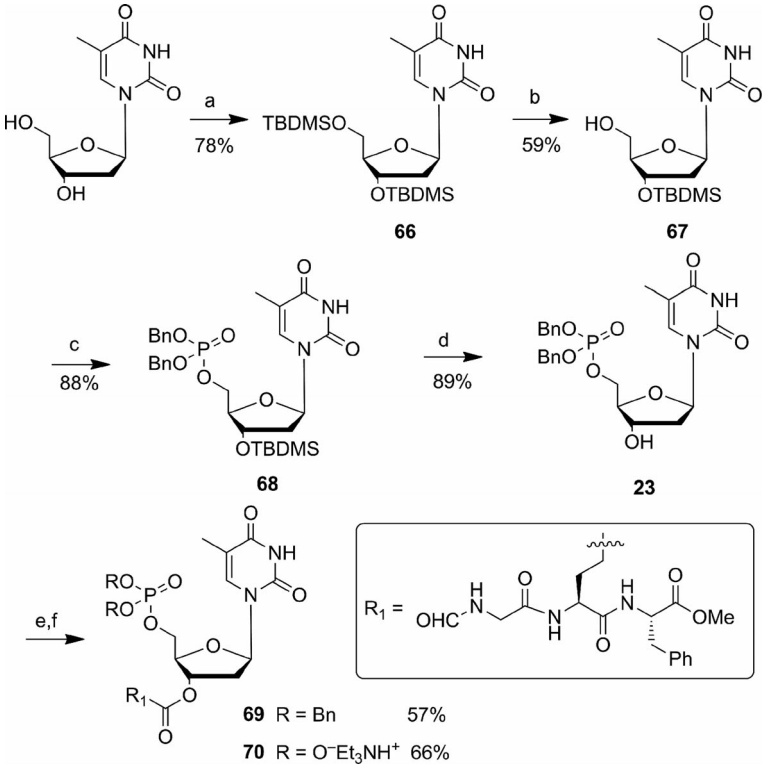
Scheme 6. Synthesis of 3′-oxymethyleneoxy ester–peptide conjugate of dTMP **62**. Reagents and conditions: (a) (i) SO_2Cl_2, CH_2Cl_2, 0 °C to room temp., 2 h, (ii) peptide-CO_2H **5a**, DBU, CH_2Cl_2, room temp., 24 h; (b) $Et_3NH{\cdot}3HF$, THF, room temp., 36 h; (c) (i) dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole (0.45 M in MeCN), CH_2Cl_2, room temp., 12 h, (ii) H_2O_2, –40 °C to room temp., 2 h; (d) $Pd(OH)_2$/C (20%), H_2, $NaHCO_3$, EtOH/H_2O, room temp., 1.5 h.



Scheme 7. Synthesis of 3′-carboxy ester–peptide conjugate of dTMP **65**. Reagents and conditions: (a) peptide-CO_2H **4h**, DCC, DMAP, CH_2Cl_2/DMF (5:1), room temp., 24 h; (b) Pd/C (10%; Degussa), H_2, MeOH, room temp., 24 h; (c) TFA, thioanisole, H_2O, room temp., 5 h.

Experimental Section

General Information: For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out under an argon or nitrogen atmosphere in oven-dried glassware (135 °C). Reaction temperatures are reported as bath temperatures. Precoated aluminum sheets (254 nm) were used for TLC, and compounds were visualized with UV light (λ = 254 nm). Products were purified by flash chromatography on ICN silica gel 63–200, 60 Å. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded with Bruker Avance 300, 500, or 600 MHz spectrometers. For final compounds, ^1H and ^{13}C resonance assignments were made using 2D NMR correlation experiments (COSY, gHSQC and gHMBC). For the sake of clarity, the NMR signals of protons and carbons for sugar and base moieties are indicated with and without a prime, respectively. Chemical shifts were referenced to residual solvent signals at $\delta_{H/C}$ 7.26/77.00 ($CDCl_3$), 3.31/49.10 ([D_4]methanol), and 2.50/39.50 ([D_6]DMSO) ppm relative to tetramethylsilane. Coupling constants are expressed in Hertz (Hz). Splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). High-resolution mass spectra were acquired with a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min, and spectra were obtained in positive or negative ionization mode with a resolution of 15000 (FWHM; full width at half maximum), using leucine enkephalin as lock mass. All the methods used MeCN/H_2O gradients. Water contained either TFA (0.1%) or NH_3 (0.1%). All final compounds were purified by preparative RP-HPLC (Xbridge™ Prep C18 5 μm OBD 19 × 150 mm column).



Scheme 8. Synthesis of 3′-carboxy ester peptide conjugate of dTMP **70**. Reagents and conditions: (a) TBDMSCl, Im (imidazole), dry DMF; (b) TFA/H_2O (10:1), CH_2Cl_2; (c) (i) dibenzyl *N,N*-diisopropylphosphoramidite, tetrazole (0.45 M in MeCN), CH_2Cl_2, 0 °C to room temp., 12 h, (ii) H_2O_2, –25 °C to room temp., 2 h; (d) $Et_3N{\cdot}3HF$, THF, 24 h; (e) peptide-CO_2H **5b**, DCC, DMAP, DMF, CH_2Cl_2, room temp., 24 h; (f) Pd/C (10%; Degussa), H_2, THF, room temp., 24 h.

General Procedures for Oligopeptides Synthesis

Method A: DCC (1.3 equiv.) was added to a solution of *N*-Boc or *N*-formyl amino acid (1 equiv.) and *N*-hydroxysuccinimide (1.2 equiv.) in dry THF (ca. 4 mL/mmol) at 0 °C. The mixture was stirred for 0.5 h at 0 °C, then for 2–6 h (monitoring by TLC) at room temp. under an argon atmosphere. The resulting dicyclohexylurea (DCU) was removed by filtration, and the filtrate was concentrated in vacuo to give the corresponding activated ester. This compound was then dissolved in dry THF (2 mL/mmol), and a suspension of a second amino acid (1.2 equiv.) and $NaHCO_3$ (4 equiv.) in water (2 mL/mmol) was then added to this solution at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temp. for 16 h, then it was cooled to 0 °C and neutralized by the addition of HCl (1 N). The aqueous layer was extracted with ethyl acetate (3 ×), and the combined organic extracts were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude residue was purified by column chromatography on silica gel (gradient: CH_2Cl_2/MeOH, 98:2, v/v; 96:4, v/v; 94:6, v/v) to give the desired compound.

Method B: A minor modification of Method A was made during the work-up stage when the carboxyl group at the C-terminal in the peptides was protected as an ester or amide. Thus, after amide coupling with the activated ester, the reaction mixture was diluted with $NaHCO_3$ (10% aq.) and then extracted with ethyl acetate. This made the purification step easier by removing the acidic by-products.

***N*-For-L-Met-L-Glu(OBn)OMe (1a):** Following Method B, dipeptide **1a** was obtained as a white solid (1.01 g, 87%) in two steps, starting from *N*-For-L-Met-OH (0.5 g, 2.82 mmol), *N*-hydroxysuccinimide (0.39 g, 3.38 mmol), and DCC (0.76 g, 3.67 mmol) in THF (20 mL), and H-L-Glu(OBn)OMe·TFA salt [1.18 g, 3.24 mmol; prepared from Boc-L-Glu(OBn)OMe and TFA in CH_2Cl_2 with thioanisole (1 equiv.)] and $NaHCO_3$ (0.95 g, 11.3 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.16 (s, 1 H, C*H*O), 7.35–7.32 (m, 5 H, Ar-*H*), 7.03 (d, J = 7.7 Hz, 1 H, N*H*-Glu), 6.47 (d, J = 7.5 Hz, 1 H, N*H*-Met), 5.12 (s, 2 H, OCH_2Ph), 4.77–4.70 (m, 1 H, αC*H*-Met), 4.64–4.59 (m, 1 H, αC*H*-Glu), 3.74 (s, 3 H, OCH_3), 2.64–2.55 (m, 2 H, γCH_2-Met), 2.52–2.43 (m, 2 H, γCH_2-Glu), 2.29–1.96 (unresolved m, 7 H, βCH_2-Glu, SCH_3, and βCH_2-Met) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7 (δCO-Glu), 171.8 (αCO-Glu), 170.7 (CO-Met), 160.9 (CHO), 135.8 (1C-Ph), 128.7 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 66.8 (OCH_2Ph), 52.7 (OCH_3), 52.0 (αC-Glu), 50.7 (αC-Met), 31.8 (βC-Met), 30.4 (γC-Glu), 29.9 (γC-Met), 27.0 (βC-Glu), 15.2 (SCH_3) ppm. HRMS: calcd. for $C_{19}H_{26}N_2O_6S$ $[M - H]^-$ 411.1584; found 411.1584.

***N*-For-L-Met-L-Glu(OBn)-NH_2 (1b):** Following Method B, dipeptide **1b** was obtained as a white solid (0.76 g, 68%) in two steps, starting from *N*-For-L-Met-OH (0.5 g, 2.82 mmol), *N*-hydroxysuccinimide (0.39 g, 3.38 mmol), and DCC (0.76 g, 3.67 mmol) in THF (20 mL), and H-L-Glu(OBn)-NH_2·TFA salt [1.09 g, 3.24 mmol; prepared from Boc-L-Glu(OBn)-NH_2 and TFA in CH_2Cl_2 with thioanisole (1 equiv.)] and $NaHCO_3$ (0.95 g, 11.3 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, [D_6]-DMSO): δ = 8.32 (d, J = 8.2 Hz, 1 H, N*H*-Met), 8.05 (d, J = 8.0 Hz, 1 H, N*H*-Glu), 8.02 (s, 1 H, C*H*O), 7.38–7.34 (m, 5 H, Ar-*H*), 7.30 (br. s, 1 H, N*H*-CONH$_2$), 7.09 (br. s, 1 H, N*H*-CONH$_2$), 5.08 (s, 2 H, OCH_2Ph), 4.43–4.36 (m, 1 H, αC*H*-Met), 4.24–4.16 (m, 1 H, αC*H*-Glu), 2.44 (t, J = 7.9 Hz, 2 H, γCH_2-Met), 2.36 (t, J = 7.8 Hz, 2 H, γCH_2-Glu), 2.02 (s, 3 H, SCH_3), 1.98–1.86 (m, 2 H, βCH_2-Glu), 1.81–1.71 (m, 2 H, βCH_2-Met) ppm. ^{13}C NMR

(75 MHz, [D$_6$]DMSO): δ = 172.7 (αCO-Glu), 172.1 (δCO-Glu), 170.7 (CO-Met), 161.1 (CHO), 136.2 (1C-Ph), 128.4 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 65.5 (OCH$_2$Ph), 51.6 (αC-Glu), 50.6 (αC-Met), 31.9 (βC-Met), 30.1 (γC-Glu), 29.3 (γC-Met), 27.1 (βC-Glu), 14.6 (SCH$_3$) ppm. HRMS: calcd. for $C_{18}H_{25}N_3O_5S$ [M + Na]$^+$ 418.1407; found 418.1407.

***N*-For-Gly-L-Glu(OBn)OH (3a):** Following Method A, dipeptide **3a** was obtained as a white solid (2.47 g, 79%) in two steps, starting from *N*-For-Gly-OH (1.0 g, 9.70 mmol), *N*-hydroxysuccinimide (1.34 g, 11.6 mmol), and DCC (2.6 g, 12.6 mmol) in THF (30 mL), and H-L-Glu(OBn)-OH·TFA salt [3.92 g, 11.2 mmol; prepared from Boc-L-Glu(OBn)-OH and TFA in CH_2Cl_2 with thioanisole (1 equiv.)] and $NaHCO_3$ (3.26 g, 38.8 mmol) in 1:1 THF/H_2O (60 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.73 (br. s, 1 H, CO$_2$*H*), 8.23–8.21 (m, 2 H, 2 N*H*), 8.07 (s, 1 H, C*H*O), 7.37–7.34 (m, 5 H, Ar-*H*), 5.09 (s, 2 H, OC*H*$_2$Ph), 4.30–4.23 (m, 1 H, αC*H*-Glu), 3.78 (app t, *J* = 5.3 Hz, 2 H, αC*H*$_2$-Gly), 2.43 (t, *J* = 7.7 Hz, 2 H, γC*H*$_2$-Glu), 2.10–1.78 (m, 2 H, βC*H*$_2$-Glu), 1.81–1.71 (m, 2 H, βC*H*$_2$-Met) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.0 (αCO-Glu), 172.1 (δCO-Glu), 168.5 (CO-Gly), 161.4 (CHO), 136.2 (1C-Ph), 128.4 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 65.5 (OCH$_2$Ph), 51.1 (αC-Glu), 40.3 (αC-Gly), 30.0 (γC-Glu), 26.3 (βC-Glu) ppm. HRMS: calcd. for $C_{15}H_{18}N_2O_6$ [M – H]$^-$ 321.1092; found 321.1090.

***N*-For-Gly-L-Glu(O*t*Bu)OH (3b):** Following Method A, dipeptide **3b** was obtained as a white solid (2.79 g, 84%) in two steps, starting from *N*-For-L-Gly-OH (1.00 g, 9.70 mmol), *N*-hydroxysuccinimide (1.34 g, 11.6 mmol), and DCC (2.60 g, 12.6 mmol) in THF (16 mL), and H-L-Glu(O*t*Bu)-OH (2.46 g, 12.1 mmol) and $NaHCO_3$ (2.24 g, 26.7 mmol, 2.2 equiv.) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, [D$_4$]methanol): δ = 8.18 (s, 1 H, C*H*O), 4.49–4.45 (m, 1 H, αC*H*-Glu), 3.99 (br. s, 2 H, αC*H*$_2$-Gly), 2.34 (t, *J* = 7.5 Hz, 2 H, γC*H*$_2$-Glu), 2.21–2.09 (m, 1 H, βC*H*$_2$-Glu), 1.98–1.88 (m, 1 H, βC*H*$_2$-Glu), 1.43 [s, 9 H, C(C*H*$_3$)$_3$] ppm. ^{13}C NMR (75 MHz, [D$_4$]methanol): δ = 173.0 (CO-Glu), 172.1 (CO-Glu), 169.3 (CO-Gly), 162.6 (CHO), 80.2 (1C-*t*Bu), 51.2 (αC-Glu), 40.2 (αC-Gly), 30.8 (γC-Glu), 26.7 (*t*Bu), 26.2 (βC-Glu) ppm. HRMS: calcd. for $C_{12}H_{19}N_2O_6$ [M – H]$^-$ 287.1248; found 287.1245.

***N*-For-Gly-L-β-amino-Ala(Fmoc)OH (3c):** Following Method A, dipeptide **3c** was obtained as a white solid (1.54 g, 70.3%) in two steps, starting from *N*-For-Gly-OH (0.55 g, 5.33 mmol), *N*-hydroxysuccinimide (0.74 g, 6.40 mmol), and DCC (1.43 g, 6.93 mmol) in THF (20 mL) and H-L-β-amino-Ala(Fmoc)-OH·TFA salt [2.70 g, 6.13 mmol; prepared from Boc-L-β-amino-Ala(Fmoc)-OH and TFA in CH_2Cl_2 with thioanisole (1 equiv.)] and $NaHCO_3$ (1.79 g, 21.4 mmol) in 1:1 THF/H_2O (50 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.19 (br. s, 1 H, CO$_2$*H*), 8.24 (t, *J* = 5.0 Hz, 1 H, N*H*), 8.17 (d, *J* = 7.9 Hz, 1 H, N*H*), 8.08 (s, 1 H, C*H*O), 7.89 (d, *J* = 7.4 Hz, 2 H, Ar*H*-Fmoc), 7.69 (d, *J* = 7.3 Hz, 2 H, Ar*H*-Fmoc), 7.44–7.31 (m, 4 H, Ar*H*-Fmoc), 4.35–4.22 (unresolved m, 4 H, α*H*-β-amino-Ala, OC*H*$_2$-Fmoc, and C*H*-Fmoc), 3.80 (d, *J* = 5.8 Hz, 2 H, αC*H*$_2$-Gly), 3.50–3.30 (m, 2 H, βC*H*$_2$-β-amino-Ala merged with DMSO) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.5 (CO-β-amino-Ala), 168.5 (CO-Gly), 161.4 (CHO), 156.3 (OCONH), 143.8 (Ar-C), 140.7 (Ar-C), 127.6 (Ar-C), 127.1 (Ar-C), 125.2 (Ar-C), 120.1 (Ar-C), 65.6 (OCH$_2$-Fmoc), 52.3 (αC-β-amino-Ala), 46.6 (CH-Fmoc), 41.5 (αC-Gly), 30.7 (βC-β-amino-Ala) ppm. HRMS: calcd. for $C_{21}H_{21}N_3O_6$ [M – H]$^-$ 410.1357; found 410.1357.

***N*-For-Gly-Lys(Boc)OH (3d):** Following Method A, dipeptide **3d** was obtained as a white solid (2.60 g, 81%) in two steps, starting from *N*-For-Gly-OH (1.0 g, 9.70 mmol), *N*-hydroxysuccinimide (1.34 g, 11.6 mmol), and DCC (2.60 g, 12.6 mmol) in THF (35 mL), and H-L-Lys(Boc)OH (2.87 g, 11.6 mmol) and $NaHCO_3$ (2.44 g, 29.1 mmol) in 1:1 THF/H_2O (40 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.16 (br. s, 1 H, CO$_2$*H*), 8.20–8.14 (m, 2 H, 2 N*H*), 8.06 (s, 1 H, C*H*O), 6.76 (t, *J* = 4.9 Hz, 1 H, εN*H*-Lys), 4.19–4.12 (m, 1 H, α*H*-Lys), 3.79 (d, *J* = 7.5 Hz, 2 H, α*H*-Gly), 2.91–2.85 (m, 2 H, εC*H*$_2$-Lys), 1.71–1.52 (m, 2 H, βC*H*$_2$-Lys), 1.39–1.33 (m, 11 H, *t*Bu and δC*H*$_2$-Lys), 1.31–1.22 (m, 2 H, γC*H*$_2$-Lys) ppm. HRMS: calcd. for $C_{14}H_{25}N_3O_6$ [M + Na]$^+$ 354.1636; found 354.1635.

***N*-For-L-Met-L-Lys(Boc)OH (3e):** Following Method A, dipeptide **3e** was obtained as a white solid (1.25 g, 84%), in two steps, starting from *N*-For-L-Met-OH (0.65 g, 3.67 mmol), *N*-hydroxysuccinimide (0.51 g, 4.40 mmol), and DCC (0.98 g, 4.77 mmol) in THF (20 mL), and H-L-Lys(Boc)-OH (0.99 g, 4.03 mmol) and $NaHCO_3$ (1.23 g, 14.7 mmol) in 1:1 THF/H_2O (40 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.56 (br. s, 1 H, CO$_2$*H*), 8.28 (d, *J* = 8.3 Hz, 1 H, N*H*-Met), 8.24 (d, *J* = 7.6 Hz, 1 H, αN*H*-Lys), 8.00 (s, 1 H, C*H*O), 6.76 (t, *J* = 5.3 Hz, 1 H, εN*H*-Lys), 4.50–4.43 (m, 1 H, α*H*-Met), 4.15–4.08 (m, 1 H, α*H*-Lys), 2.91–2.85 (m, 2 H, εC*H*$_2$-Lys), 2.44 (t, *J* = 8.0 Hz, 2 H, γC*H*$_2$-Met), 2.03 (s, 3 H, -SC*H*$_3$), 1.95–1.77 (m, 2 H, βC*H*$_2$-Met), 1.75–1.56 (m, 2 H, βC*H*$_2$-Lys), 1.36–1.21 (m, 13 H, *t*Bu, δC*H*$_2$-Lys, and γC*H*$_2$-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.4 (CO-Lys), 170.7 (CO-Met), 160.8 (CHO), 155.5 (OCONH), 77.2 (1C of *t*Bu), 51.9 (αC-Lys), 50.1 (αC-Met), 39.6 (εC-Lys), 32.4 (βC-Met), 30.4 (βC-Lys), 29.2 (γC-Met), 29.1 (δC-Lys), 28.2 (CH$_3$-*t*Bu), 22.8 (γC-Lys), 14.6 (SC*H*$_3$) ppm. HRMS: calcd. for $C_{17}H_{31}N_3O_6S$ [M – H]$^-$ 404.1861; found 404.1861.

***N*-For-L-Met-L-Lys(Boc)OMe (3f):** Following Method B, dipeptide **3f** was obtained as a white solid (1.02 g, 86%), in two steps, starting from *N*-For-L-Met-OH (0.5 g, 2.82 mmol), *N*-hydroxysuccinimide (0.39 g, 3.39 mmol), and DCC (0.76 g, 3.67 mmol) in THF (18 mL), and H-L-Lys(Boc)OMe·HCl salt (0.92 g, 3.10 mmol) and $NaHCO_3$ (0.83 g, 9.87 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.39 (d, *J* = 7.2 Hz, 1 H, αN*H*-Lys), 8.29 (d, *J* = 8.2 Hz, 1 H, N*H*-Met), 8.01 (s, 1 H, C*H*O), 6.76 (t, *J* = 5.4 Hz, 1 H, εN*H*-Lys), 4.51–4.44 (m, 1 H, α*H*-Met), 4.22–4.16 (m, 1 H, α*H*-Lys), 3.62 (s, 3 H, OC*H*$_3$), 2.91–2.85 (m, 2 H, εC*H*$_2$-Lys), 2.44 (t, *J* = 7.9 Hz, 2 H, γC*H*$_2$-Met), 2.04 (s, 3 H, SC*H*$_3$), 1.93–1.77 (m, 2 H, βC*H*$_2$-Met), 1.75–1.58 (m, 2 H, βC*H*$_2$-Lys), 1.37 (s, 9 H, *t*Bu), 1.35–1.21 (m, 4 H, δC*H*$_2$-Lys and γC*H*$_2$-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.4 (CO-Lys), 170.9 (CO-Met), 160.8 (CHO), 155.5 (OCONH), 77.3 (1C of *t*Bu), 52.0 (αC-Lys), 51.8 (OCH$_3$), 50.0 (αC-Met), 39.6 (εC-Lys), 32.3 (βC-Met), 30.3 (βC-Lys), 29.2 (γC-Met), 29.0 (δC-Lys), 28.2 (CH$_3$-*t*Bu), 22.7 (γC-Lys), 14.6 (SCH$_3$) ppm. HRMS: calcd. for $C_{18}H_{33}N_3O_6S$ [M – H]$^-$ 418.2017; found 418.2022.

Boc-L-Ala-L-Lys(Cbz)OH (11): Following Method A, dipeptide **11** was obtained as a white solid (1.63 g, 68%), in two steps, starting from Boc-L-Ala-OH (1.0 g, 5.29 mmol), *N*-hydroxysuccinimide (0.73 g, 6.35 mmol), and DCC (1.42 g, 6.88 mmol) in THF (25 mL), and H-L-Lys(Cbz)OH (1.70 g, 6.08 mmol) and $NaHCO_3$ (1.78 g, 21.2 mmol) in 1:1 THF/H_2O (50 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.49 (br. s, 1 H, CO$_2$*H*), 7.80 (d, *J* = 7.6 Hz, 1 H, αN*H*-Lys), 7.29–7.23 (m, 5 H, Ar-*H*), 7.10 (t, *J* = 5.9 Hz, 1 H, εN*H*-Lys), 6.77 (d, *J* = 7.5 Hz, 1 H, N*H*-Ala), 4.91 (s, 2 H, OC*H*$_2$Ph), 4.10–4.03 (m, 1 H, α*H*-Lys), 3.93–3.86 (m, 1 H, α*H*-Ala), 2.90–2.85 (m, 2 H, εC*H*$_2$-Lys), 1.64–1.41 (m, 2 H, βC*H*$_2$-Lys), 1.35–1.20 (m, 11 H, CH$_3$-*t*Bu and δC*H*$_2$-Lys), 1.16–0.93 (m, 5 H, C*H*$_3$-Ala and γC*H*$_2$-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO): δ = 173.6 (CO-Lys), 172.9 (CO-Ala), 156.2 (OCONH), 155.1 (OCONH), 137.4 (1C-Ph), 128.5 (Ar-C), 127.8 (Ar-C), 78.2

(1C of *t*Bu), 65.2 (-OCH_2Ph), 51.7 (αC-Lys), 49.6 (αC-Ala), 40.2 (εC-Lys), 30.9 (βC-Lys), 29.1 (δC-Lys), 28.3 (CH_3-*t*Bu), 22.6 (γC-Lys), 18.2 (CH_3-Ala) ppm. HRMS: calcd. for $C_{22}H_{33}N_3O_7$ [M – H]$^-$ 450.2245; found 450.2236.

***N*-For-Met-L-Glu-OMe (2a):** Pd/C (10%; Degussa; 0.5 g, 50% w/w) was added to a stirred solution of **1a** (1.00 g, 2.44 mmol) in EtOH, and the mixture was hydrogenated at atmospheric pressure using a balloon filled with H_2 for 16 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The resulting crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 94:6, v/v) to give compound **2a** (0.75 g, 96%) as a white solid. ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 12.15 (br. s, 1 H, CO_2H), 8.43 (t, J = 7.4 Hz, 1 H, N*H*-Glu), 8.30 (d, J = 7.9 Hz, 1 H, N*H*-Met), 8.01 (s, 1 H, C*H*O), 4.89–4.41 (m, 1 H, α*H*-Met), 4.31–4.23 (m, 1 H, α*H*-Glu), 3.62 (s, 3 H, OCH_3), 2.44 (t, J = 7.9 Hz, 2 H, γCH_2-Met), 2.29 (t, J = 7.5 Hz, 2 H, γCH_2-Glu), 2.04 (s, 3 H, -SCH_3), 2.01–1.72 (unresolved m, 4 H, βCH_2-Glu and βCH_2-Met) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 173.6 (δCO-Glu), 172.0 (αCO-Glu), 171.0 (CO-Met), 160.9 (CHO), 51.9 (OCH_3), 51.2 (αC-Glu), 50.1 (αC-Met), 32.1 (βC-Met), 29.8 (γC-Glu), 29.2 (γC-Met), 25.9 (βC-Glu), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{11}H_{20}N_2O_6S$ [M – H]$^-$ 319.0969; found 319.0975.

***N*-For-Met-L-Glu-NH_2 (2b):** Following a similar procedure to that used for the synthesis of compound **2a**, **1b** (0.76 g, 2.44 mmol) was hydrogenated in the presence of Pd/C (10%; Degussa; 0.38 g, 50% w/w) in EtOH, and the resulting product was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:2, v/v; 97:4, v/v; 94:8, v/v) to give **2b** (0.51 g, 86%) as a white solid. ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 12.09 (br. s, 1 H, CO_2H), 8.33 (d, J = 7.9 Hz, 1 H, N*H*-Met), 8.04–8.01 (m, N*H*-Glu and C*H*O), 7.29 (br. s, 1 H, N*H*-$CONH_2$), 7.07 (br. s, 1 H, N*H*-$CONH_2$), 4.44–4.37 (m, 1 H, α*H*-Met), 4.21–4.14 (m, 1 H, α*H*-Glu), 2.44 (t, J = 7.9 Hz, 2 H, γCH_2-Met), 2.29 (t, J = 7.6 Hz, 2 H, γCH_2-Glu), 2.03 (s, 3 H, SCH_3), 1.97–1.85 (m, 2 H, βCH_2-Glu), 1.81–1.71 (m, 2 H, βCH_2-Met) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 174.0 (δCO-Glu), 172.9 (αCO-Glu), 170.6 (CO-Met), 161.2 (CHO), 51.8 (αC-Glu), 50.6 (αC-Met), 31.9 (βC-Met), 30.3 (γC-Glu), 29.3 (γC-Met), 27.2 (βC-Glu), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{11}H_{19}N_3O_5S$ [M – H]$^-$ 304.0972; found 304.0975.

***N*-For-L-Met-L-Lys(NH_2·TFA)-OMe (8):** Trifluoroacetic acid (1 mL) was slowly added to a stirred solution of **3f** (162.9 mg, 0.388 mmol) and thioanisole (0.046 mL, 0.388 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was then slowly warmed to room temp. and stirred for 4 h. All the volatiles were removed under reduced pressure, and the resulting residue was coevaporated with toluene (3 ×) to give compound **8** (168.3 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

***N*-For-Gly-L-Glu(OBn)-L-Ala-OMe (4a):** Following Method B, tripeptide **4a** was obtained as a white solid (2.04 g, 79%) in two steps, starting from **3a** (2.04 g, 6.33 mmol), *N*-hydroxysuccinimide (0.87 g, 7.59 mmol), and DCC (1.70 g, 8.23 mmol) in THF (36 mL), and H-L-Ala-OMe·HCl salt (1.02 g, 7.28 mmol) and $NaHCO_3$ (2.13 g, 25.32 mmol) in 1:1 THF/H_2O (40 mL). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 8.43 (d, J = 6.8 Hz, 1 H, N*H*-Ala), 8.21 (t, J = 5.2 Hz, 1 H, N*H*-Gly), 8.10 (d, J = 8.1 Hz, 1 H, N*H*-Glu), 8.06 (s, 1 H, C*H*O), 7.38–7.32 (m, 5 H, Ar-*H*), 5.09 (s, 2 H, OCH_2Ph), 4.40–4.33 (m, 1 H, αC*H*-Glu), 4.30–4.21 (m, 1 H, αC*H*-Ala), 3.77 (d, J = 7.3 Hz, 2 H, αCH_2-Gly), 3.59 (s, 3 H, OCH_3), 2.41 (t, J = 8.1 Hz, 2 H, γCH_2-Glu), 2.02–1.75 (m, 2 H, βCH_2-Glu), 1.29 (d, J = 7.3 Hz, 3 H, CH_3-Ala) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 172.8 (δCO-Glu), 172.2 (CO-Ala), 170.8 (αCO-Glu), 168.3 (CO-Gly), 161.4 (CHO), 136.2 (1C Ph), 128.4 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 65.5 (OCH_2Ph), 51.8 (OCH_3), 51.2 (αC-Glu), 47.6 (αC-Ala), 40.5 (αC-Gly), 29.8 (γC-Glu), 27.5 (βC-Glu), 16.6 (CH_3-Ala) ppm. HRMS: calcd. for $C_{19}H_{25}N_3O_7$ [M + H]$^+$ 408.1765; found 408.1765.

***N*-For-Gly-L-Glu(O*t*Bu)-L-Phe-OMe (4b):** Following Method A, tripeptide **4b** was obtained as a white solid (2.29 g, 69%) in two steps, starting from **3b** (2.11 g, 7.32 mmol), *N*-hydroxysuccinimide (1.01 g, 8.78 mmol), and DCC (1.96 g, 9.51 mmol) in THF (14 mL), and H-L-Phe-OMe·HCl salt (2.00 g, 9.27 mmol) and $NaHCO_3$ (1.72 g, 20.5 mmol, 2.2 equiv.) in 1:1 THF/H_2O (40 mL). ^{1}H NMR (300 MHz, [D_4]methanol): δ = 8.18 (s, 1 H, C*H*O), 7.29–7.20 (m, 5 H, Ar-*H*), 4.67 (dd, J = 8.5, 5.6 Hz, 1 H, αC*H*-Phe), 4.40 (dd, J = 8.3, 5.3 Hz, 1 H, αC*H*-Glu), 3.92 (br. s, 2 H, αCH_2-Gly), 3.70 (s, 3 H, OCH_3), 3.21–3.15 (m, 1 H, βCH_2-Phe), 3.06–2.98 (m, 1 H, βCH_2-Phe), 2.31–2.26 (m, 2 H, γCH_2-Glu), 2.04–2.00 (m, 1 H, βCH_2-Glu), 1.88–1.85 (m, 1 H, βCH_2-Glu), 1.46 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (75 MHz, [D_4]methanol): δ = 172.2 (δCO-Glu), 171.6 (CO), 171.4 (CO), 169.1 (CO), 162.5 (CHO), 136.3 (1C-Ph), 128.5 (Ar-C), 127.8 (Ar-C), 126.2 (Ar-C), 53.5, 52.0, 51.0, 40.3, 36.5, 30.7 (γC-Glu), 26.6 (βC-Glu), 26.5 (*t*Bu) ppm. HRMS: calcd. for $C_{22}H_{32}N_3O_7$ [M + H]$^+$ 450.2234; found 450.2233.

***N*-For-Gly-L-β-amino-Ala(Fmoc)-L-Phe-OMe (4c):** Following Method B, tripeptide **4c** was obtained as a white solid (1.42 g, 72.8%) in two steps, starting from **3c** (1.4 g, 3.40 mmol), *N*-hydroxysuccinimide (0.47 g, 4.09 mmol), and DCC (0.91 g, 4.42 mmol) in THF (25 mL), and H-L-Phe-OMe·HCl salt (0.84 g, 3.91 mmol) and $NaHCO_3$ (1.14 g, 13.6 mmol) in 1:1 THF/H_2O (50 mL). ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 8.41 (d, J = 7.6 Hz, 1 H, N*H*-Phe), 8.24 (t, J = 4.9 Hz, 1 H, N*H*-Gly), 8.09 (s, 1 H, CHO), 8.02 (d, J = 8.1 Hz, 1 H, N*H*-β-amino-Ala), 7.88 (d, J = 7.6 Hz, 2 H, Ar*H*-Fmoc), 7.69 (d, J = 7.5 Hz, 2 H, Ar*H*-Fmoc), 7.43–7.15 (m, 9 H, Ar*H*-Fmoc and Ar*H*-Phe), 7.17 (t, J = 5.0 Hz, 1 H, βN*H*-β-amino-Ala), 4.51–4.47 (m, 1 H, α*H*-Phe), 4.55–4.42 (m, 1 H, α*H*-β-amino-Ala), 4.30–4.28 (m, 1 H, OCH_2-Fmoc), 4.24–4.21 (m, 1 H, C*H*-Fmoc), 3.79 (d, J = 5.7 Hz, 2 H, αCH_2-Gly), 3.56 (s, 3 H, OCH_3), 3.35–3.15 (m, 2 H, βCH_2-β-amino-Ala), 3.06–2.93 (m, 2 H, βCH_2-Phe) ppm. ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 171.7 (CO-Phe), 169.7 (CO-β-amino-Ala), 168.5 (CO-Gly), 161.6 (CHO), 156.3 (OCONH), 143.9 (1C Fmoc), 140.8 (1C Fmoc), 137.1 (1C Ph), 129.2 (Ar-C), 129.1 (Ar-C), 128.3 (Ar-C), 127.7 (Ar-C), 127.4 (Ar-C), 127.2 (Ar-C), 126.7 (Ar-C), 125.3 (Ar-C), 121.5 (Ar-C), 120.1 (Ar-C), 65.7 (OCH_2-Fmoc), 53.8 (αC-Phe), 52.7 (αC-β-amino-Ala), 52.0 (OCH_3), 46.7 (CH-Fmoc), 42.3 (βC-Phe), 40.7 (αC-Gly), 36.6 (βC-β-amino-Ala) ppm. HRMS: calcd. for $C_{31}H_{32}N_4O_7$ [M + H]$^+$ 573.2343; found 573.2346.

***N*-For-Gly-L-Lys(Boc)-L-Phe-OMe (4d):** Following Method B, tripeptide **4d** was obtained as a white solid (1.82 g, 72%) in two steps, starting from **3d** (1.7 g, 5.13 mmol), *N*-hydroxysuccinimide (0.71 g, 6.16 mmol), and DCC (1.38 g, 6.67 mmol) in THF (35 mL), and H-L-Phe-OMe·HCl salt (1.33 g, 6.16 mmol) and $NaHCO_3$ (1.72 g, 20.52 mmol) in 1:1 THF/H_2O (50 mL). ^{1}H NMR (500 MHz, [D_6]-DMSO): δ = 8.37 (d, J = 7.4 Hz, 1 H, N*H*-Phe), 8.19 (t, J = 5.2 Hz, 1 H, N*H*-Gly), 8.06 (s, 1 H, C*H*O), 7.97 (d, J = 8.1 Hz, 1 H, N*H*-Lys), 7.30–7.19 (m, 5 H, Ar-*H*), 6.73 (t, J = 5.1 Hz, 1 H, εN*H*-Lys), 4.49–4.41 (m, 1 H, α*H*-Phe), 4.31–4.24 (m, 1 H, α*H*-Lys), 3.76 (d, J = 5.7 Hz, 2 H, α*H*-Gly), 3.57 (s, 3 H, OCH_3), 3.06–2.92 (m, 2 H, βCH_2-Phe), 2.89–2.83 (m, 2 H, εCH_2-Lys), 1.61–1.43 (m, 2 H, βCH_2-Lys), 1.37–1.29 (m, 11 H, *t*Bu and δCH_2-Lys), 1.26–1.13 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (125 MHz, [D_6]DMSO):

δ = 171.7 (CO-Phe), 171.6 (CO-Lys), 168.0 (CO-Gly), 161.4 (CHO), 155.5 (OCONH), 137.1 (1C-Ph), 129.0 (Ar-C), 128.2 (Ar-C), 126.5 (Ar-C), 77.3 (1C of *t*Bu), 53.6 (αC-Phe), 52.1 (αC-Lys), 51.8 (OCH_3), 40.4 (αC-Gly), 39.6 (εC-Lys), 36.5 (βC-Phe), 31.9 (βC-Lys), 29.3 (δC-Lys), 28.3 (CH_3-*t*Bu), 22.4 (γC-Lys) ppm. HRMS: calcd. for $C_{24}H_{36}N_4O_7$ $[M + H]^+$ 493.2656; found 493.2659.

***N*-For-L-Met-L-Lys(Boc)-L-Ala-OMe (4e):** Following Method B, tripeptide **4e** was obtained as a white solid (1.50 g, 88.5%) in two steps, starting from **3e** (1.4 g, 3.45 mmol), *N*-hydroxysuccinimide (0.48 g, 4.14 mmol), and DCC (0.93 g, 4.49 mmol) in THF (30 mL), and H-L-Ala-OMe·HCl salt (0.53 g, 3.80 mmol) and $NaHCO_3$ (0.87 g, 10.36 mmol) in 1:1 THF/H_2O (40 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.30 (t, *J* = 6.9 Hz, 1 H, N*H*), 8.27 (d, *J* = 9.4 Hz, 1 H, N*H*), 8.02–8.00 (m, 2 H, C*H*O and N*H*), 6.73 (t, *J* = 5.3 Hz, 1 H, εN*H*-Lys), 4.47–4.40 (m, 1 H, α*H*-Met), 4.29–4.20 (m, 2 H, α*H*-Lys and α*H*-Ala), 3.61 (s, 3 H, OCH_3), 2.91–2.84 (m, 2 H, εCH_2-Lys), 2.42 (t, *J* = 7.9 Hz, 2 H, γCH_2-Met), 2.02 (s, 3 H, SCH_3), 1.94–1.72 (m, 2 H, βCH_2-Met), 1.69–1.49 (m, 2 H, βCH_2-Lys), 1.40–1.31 (m, 11 H, CH_3-*t*Bu and δCH_2-Lys), 1.29–1.20 (m, 5 H, CH_3-Ala and γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.9 (CO-Ala), 171.4 (CO-Lys), 170.5 (CO-Met), 160.9 (CHO), 155.5 (OCONH), 77.3 (1C of *t*Bu), 52.2 (αC-Lys), 51.8 (OCH_3), 50.3 (αC-Met), 47.5 (αC-Ala), 39.8 (εC-Lys), 32.2 (βC-Met), 31.7 (βC-Lys), 29.3 (γC-Met), 29.2 (δC-Lys), 28.2 (CH_3-*t*Bu), 22.5 (γC-Lys), 16.8 (CH_3-Ala), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{21}H_{38}N_4O_7S$ $[M + Na]^+$ 513.2353; found 513.2352.

***N*-For-L-Met-L-Lys(Boc)-L-Lys(Cbz)-OMe (4f):** Following Method B, tripeptide **4f** was obtained as a white solid (1.07 g, 67%) in two steps, starting from **3e** (0.92 g, 2.27 mmol), *N*-hydroxysuccinimide (0.31 g, 2.72 mmol), and DCC (0.61 g, 2.95 mmol) in THF (20 mL), and H-L-Lys(Cbz)-OMe·HCl salt (0.86 g, 2.61 mmol) and $NaHCO_3$ (0.76 g, 9.08 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.31–8.25 (m, 2 H, 2 N*H*), 8.02–8.00 (m, 2 H, C*H*O and N*H*), 7.36–7.30 (m, 5 H, Ar-*H*), 7.22 (t, *J* = 5.1 Hz, 1 H, εN*H*-$Lys_{C\text{-}ter}$), 6.73 (t, *J* = 5.0 Hz, 1 H, εN*H*-$Lys_{N\text{-}ter}$), 5.00 (s, 2 H, OCH_2Ph), 4.47–4.40 (m, 1 H, α*H*-Met), 4.32–4.25 (m, 1 H, α*H*-$Lys_{N\text{-}ter}$), 4.22–4.15 (m, 1 H, α*H*-$Lys_{C\text{-}ter}$), 3.61 (s, 3 H, OCH_3), 2.99–2.93 (m, 2 H, εCH_2-Lys), 2.89–2.83 (m, 2 H, εCH_2-Lys), 2.41 (t, *J* = 7.9 Hz, 2 H, γCH_2-Met), 2.02 (s, 3 H, SCH_3), 1.94–1.70 (m, 2 H, βCH_2-Met), 1.69–1.44 (m, 4 H, 2 βCH_2-Lys), 1.40–1.17 (m, 17 H, CH_3-*t*Bu, 2 δCH_2-Lys, and 2 γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.4 (CO-$Lys_{C\text{-}ter}$), 171.6 (CO-Met), 170.4 (CO-$Lys_{N\text{-}ter}$), 161.0 (CHO), 156.0 (OCONH), 155.5 (OCONH), 137.3 (1C-Ph), 128.3 (Ar-C), 127.7 (Ar-C), 77.3 (1C of *t*Bu), 65.1 (OCH_2Ph), 52.3 (αC-$Lys_{N\text{-}ter}$), 51.7 (OCH_3 and αC-$Lys_{C\text{-}ter}$), 50.4 (αC-Met), 40.0 (εC-Lys), 32.1 (βC-Met), 31.9 (βC-Lys), 30.5 (βC-Lys), 29.3 (γC-Met), 29.2 (δC-Lys), 28.8 (δC-Lys), 28.2 (CH_3-*t*Bu), 22.6 (γC-Lys), 22.5 (γC-Lys), 14.5 (SCH_3) ppm. HRMS: calcd. for $C_{32}H_{51}N_5O_9S$ $[M – H]^–$ 680.3334; found 680.3336.

***N*-For-L-Met-L-Lys(Boc)-L-Phe-OMe (4g):** Following Method B, tripeptide **4g** was obtained as a white solid (1.02 g, 86%) in two steps, starting from **3e** (0.5 g, 1.23 mmol), *N*-hydroxysuccinimide (0.17 g, 1.48 mmol), and DCC (0.33 g, 1.60 mmol) in THF (18 mL), and H-L-Phe-OMe·HCl salt (0.29 g, 1.36 mmol) and $NaHCO_3$ (0.41 g, 4.93 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.14 (s, 1 H, C*H*O), 7.69 (d, *J* = 8.1 Hz, 1 H, αN*H*-Lys), 7.60–7.57 (m, 2 H, N*H*-Met and N*H*-Phe), 7.27–7.11 (m, 5 H, Ar-*H* Phe), 5.03–4.98 (m, 1 H, εN*H*-Lys), 4.96–4.88 (m, 1 H, α*H*-Met), 4.84–4.77 (m, 1 H, α*H*-Phe), 4.74–4.69 (m, 1 H, α*H*-Lys), 3.69 (s, 3 H, OCH_3), 3.11–3.01 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.47 (t, *J* = 6.8 Hz, 2 H, γCH_2-Met), 2.04 (s, 3 H, SCH_3), 2.00–1.90 (m, 2 H, βCH_2-Met), 1.79–1.63 (m, 2 H, βCH_2-Lys), 1.47–1.40 (m, 11 H, *t*Bu and δCH_2-Lys), 1.35–1.28 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0 (CO-Phe), 171.5 (CO-Lys), 171.1 (CO-Met), 161.4 (CHO), 156.2 (OCONH), 136.0 (1C of Phe), 129.2 (Ar-C), 128.6 (Ar-C), 127.2 (Ar-C), 79.1 (1C of *t*Bu), 53.7 (αC-Phe), 52.9 (αC-Lys), 52.4 (OCH_3), 50.6 (αC-Met), 40.2 (εC-Lys), 37.9 (βC-Phe), 32.8 (βC-Met and βC-Lys), 30.0 (γC-Met), 29.6 (δC-Lys), 28.5 (CH_3-*t*Bu), 22.6 (γC-Lys), 15.4 (SCH_3) ppm. HRMS: calcd. for $C_{27}H_{42}N_4O_7S$ $[M – H]^–$ 565.2701; found 565.2700.

***N*-For-L-Met-L-Lys(Boc)-L-Lys(Boc)-OH (4h):** Following Method A, tripeptide **4h** was obtained as a white solid (0.54 g, 62%) in two steps, starting from **3e** (0.56 g, 1.38 mmol), *N*-hydroxysuccinimide (0.19 g, 1.66 mmol), and DCC (0.37 g, 1.79 mmol) in THF (15 mL), and H-L-Lys(Boc)-OH (0.39 g, 1.59 mmol) and $NaHCO_3$ (0.46 g, 5.52 mmol) in 1:1 THF/H_2O (20 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.51 (s, 1 H, CO_2H), 8.28 (d, *J* = 8.0 Hz, 1 H, N*H*-Met), 8.04–8.02 (m, 3 H, 2 N*H*-Lys and C*H*O), 6.77–6.71 (m, 2 H, 2 εN*H*-Lys), 4.47–4.40 (m, 1 H, α*H*-Met), 4.28–4.24 (m, 1 H, α*H*-$Lys_{N\text{-}ter}$), 4.23–4.21 (m, 1 H, α*H*-$Lys_{C\text{-}ter}$), 2.91–2.83 (m, 4 H, 2 εCH_2-Lys), 2.42 (t, *J* = 7.8 Hz, 2 H, γCH_2-Met), 2.02 (s, 3 H, SCH_3), 1.90–1.70 (m, 2 H, βCH_2-Met), 1.69–1.48 (m, 4 H, 2 βCH_2-Lys), 1.40–1.15 (m, 26 H, 2 CH_3-*t*Bu, 2 δCH_2-Lys, and 2 γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.4 (CO-$Lys_{C\text{-}ter}$), 171.5 (CO-Met), 170.5 (CO-$Lys_{N\text{-}ter}$), 160.9 (CHO), 155.5 (2 OCONH), 77.3 (1C of *t*Bu), 52.3 (αC-$Lys_{N\text{-}ter}$), 51.8 (αC-$Lys_{C\text{-}ter}$), 50.3 (αC-Met), 39.6 (2 εC-Lys), 32.2 (βC-Met), 31.6 (βC-Lys), 30.6 (βC-Lys), 29.3 (γC-Met), 29.1 (2 δC-Lys), 28.2 (CH_3-*t*Bu), 22.6 (2 γC-Lys), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{28}H_{51}N_5O_9S$ $[M – H]^–$ 632.3334; found 632.3334.

***N*-For-L-Met-L-Lys(Boc)-L-Ala-OH (4i):** Following Method A, tripeptide **4i** was obtained as a white solid (2.04 g, 85%) in two steps, starting from **3e** (2.04 g, 5.03 mmol), *N*-hydroxysuccinimide (0.70 g, 6.04 mmol), and DCC (1.35 g, 6.54 mmol) in THF (40 mL), and H-L-Ala-OH (0.49 g, 5.53 mmol) and $NaHCO_3$ (1.69 g, 20.12 mmol) in 1:1 THF/H_2O (50 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.52 (br. s, 1 H, CO_2H), 8.28 (d, *J* = 8.0 Hz, 1 H, αN*H*-Met), 8.13 (d, *J* = 7.1 Hz, 1 H, αN*H*-Ala), 8.03–8.01 (m, 2 H, C*H*O and αN*H*-Lys), 6.73 (t, *J* = 4.4 Hz, 1 H, εN*H*-Lys), 4.47–4.40 (m, 1 H, α*H*-Met), 4.26–4.15 (m, 2 H, α*H*-Ala and α*H*-Lys), 2.89–2.84 (m, 2 H, εCH_2-Lys), 2.43 (t, *J* = 7.8 Hz, 2 H, γCH_2-Met), 2.03 (s, 3 H, -SCH_3), 1.93–1.70 (m, 2 H, βCH_2-Met), 1.63–1.48 (m, 2 H, βCH_2-Lys), 1.40–1.33 (m, 11 H, *t*Bu and δCH_2-Lys), 1.30–1.19 (m, 5 H, CH_3-Ala and γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.0 (CO_2H), 171.2 (CO-Lys), 170.5 (CO-Met), 161.0 (CHO), 155.5 (OCONH), 77.3 (1C of *t*Bu), 52.2 (αC-Lys), 50.4 (αC-Met), 47.4 (αC-Ala), 40.2 (εC-Lys), 32.2 (βC-Met), 31.7 (βC-Lys), 29.3 (γC-Met), 29.2 (δC-Lys), 28.3 (CH_3-*t*Bu), 22.6 (γC-Lys), 17.1 (CH_3-Ala), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{20}H_{36}N_4O_7S$ $[M – H]^–$ 475.2232; found 475.2231.

Boc-L-Ala-L-Lys(Cbz)-L-Ala-OMe (12): Following Method B, tripeptide **12** was obtained as a white solid (1.10 g, 75%) in two steps, starting from **11** (1.23 g, 2.72 mmol), *N*-hydroxysuccinimide (0.38 g, 3.27 mmol), and DCC (0.73 g, 3.54 mmol) in THF (20 mL), and H-L-Ala-OMe·HCl (0.44 g, 3.13 mmol) and $NaHCO_3$ (0.92 g, 10.92 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.33–7.29 (m, 5 H, Ar-*H*), 6.99–6.94 (m, 2 H, 2 N*H*), 5.28–5.22 (m, 2 H, 2 N*H*), 5.08 (s, 2 H, OCH_2Ph), 4.57–4.44 (m, 2 H, α*H*-Lys and α*H*-$Ala_{C\text{-}ter}$), 4.20–4.16 (m, 1 H, α*H*-$Ala_{N\text{-}ter}$), 3.69 (s, 3 H, OCH_3), 3.21–3.14 (m, 2 H, εCH_2-Lys), 1.89–1.61 (m, 2 H, βCH_2-Lys), 1.55–1.47 (m, 2 H, δCH_2-Lys), 1.42 (s, 9

H, CH_3-*t*Bu), 1.39–1.36 (m, 5 H, CH_3-Ala$_{C\text{-}ter}$ and γCH_2-Lys), 1.32 (d, J = 7.0 Hz, 3 H, CH_3-Ala$_{N\text{-}ter}$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3 (CO-Ala$_{C\text{-}ter}$), 172.9 (CO-Ala$_{N\text{-}ter}$), 173.3 (CO-Lys), 156.8 (OCONH), 155.7 (OCONH), 136.7 (1C-Ph), 128.6 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 80.3 (1C of *t*Bu), 66.7 (OCH_2Ph), 52.8 (αC-Lys), 52.5 (OCH_3), 50.3 (αC-Ala$_{N\text{-}ter}$), 48.2 (αC-Ala$_{C\text{-}ter}$), 40.4 (εC-Lys), 31.9 (βC-Lys), 29.3 (δC-Lys), 28.4 (CH_3-*t*Bu), 22.2 (γC-Lys), 18.4 (CH_3-Ala$_{N\text{-}ter}$), 18.0 (CH_3-Ala$_{C\text{-}ter}$) ppm. HRMS: calcd. for $C_{26}H_{40}N_4O_8$ [M + H]$^+$ 537.2919; found 537.2916.

***N*-For-Gly-L-Glu-L-Ala-OMe (5a):** Following a similar procedure to that used for the synthesis of **2a**, compound **4a** (2.00 g, 4.91 mmol) was hydrogenated in the presence of Pd/C (10%; Degussa; 0.20 g, 10% w/w) in MeOH (60 mL) to give **5a** (1.42 g, 91%) as a white solid after purification. ^{1}H NMR (300 MHz, [D$_6$]-DMSO): δ = 12.09 (br. s, 1 H, γCO_2H-Glu), 8.41 (d, J = 6.8 Hz, 1 H, NH-Ala), 8.21 (t, J = 5.2 Hz, 1 H, NH-Gly), 8.09–8.06 (m, 2 H, NH-Glu and CHO), 4.36–4.29 (m, 1 H, αCH-Glu), 4.27–4.20 (m, 1 H, αCH-Ala), 3.77 (d, J = 5.8 Hz, 2 H, αCH_2-Gly), 3.61 (s, 3 H, OCH_3), 2.25 (t, J = 8.1 Hz, 2 H, γCH_2-Glu), 1.95–1.67 (m, 2 H, βCH_2-Glu), 1.28 (d, J = 7.0 Hz, 3 H, CH_3-Ala) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 174.0 (δCO-Glu), 172.9 (CO-Ala), 171.0 (αCO-Glu), 168.3 (CO-Gly), 161.5 (CHO), 51.9 (OCH_3), 51.4 (αC-Glu), 47.6 (αC-Ala), 40.5 (αC-Gly), 29.9 (γC-Glu), 27.6 (βC-Glu), 16.7 (CH_3-Ala) ppm. HRMS: calcd. for $C_{12}H_{19}N_3O_7$ [M – H]$^-$ 316.1150; found 316.1152.

***N*-For-Gly-L-Glu-L-Phe-OMe (5b):** A solution of **4b** (0.13 g, 0.29 mmol) in CH_2Cl_2 (4 mL) was cooled to 0 °C, and trifluoroacetic acid (1.33 mL) was then added. The reaction mixture was stirred for 15 min at 0 °C, and then for 2 h at room temp. Toluene (6 mL) was then added, and the volatiles were removed in vacuo. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 98:1, v/v; 96:5, v/v; 94:7, v/v, 94:10, v/v) to give **5b** (0.11 g, quant.) as a white foam. ^{1}H NMR (300 MHz, [D$_4$]methanol): δ = 8.08 (s, 1 H, CHO), 7.21–7.10 (m, 5 H, Ar-H), 4.61–4.56 (m, 1 H, αCH-Phe), 4.35 (dd, J = 8.3, 5.6 Hz, 1 H, αCH-Glu), 3.83 (br. s, 2 H, αCH_2-Gly), 3.59 (s, 3 H, OCH_3), 3.11–3.04 (m, 1 H, βCH_2-Phe), 2.96–2.88 (m, 1 H, βCH_2-Phe), 2.26 (t, J = 8.0 Hz, 2 H, γCH_2-Glu), 1.97–1.93 (m, 1 H, βCH_2-Glu), 1.83–1.79 (m, 1 H, βCH_2-Glu) ppm. ^{13}C NMR (75 MHz, [D$_4$]-methanol): δ = 173.5 (δCO-Glu), 170.2 (CO), 169.9 (CO), 167.6 (CO), 161.1 (CHO), 134.8 (1C-Ph), 127.0 (Ar-C), 126.3 (Ar-C), 124.6 (Ar-C), 50.6, 49.5, 46.6, 38.8, 34.9, 27.8 (γC-Glu), 25.1 (βC-Glu) ppm. HRMS: calcd. for $C_{18}H_{22}N_3O_7$ [M – H]$^-$ 392.1463; found 392.1462.

***N*-For-Gly-L-β-amino-Ala-L-Phe-OMe (5c):** A solution of **4c** (0.92 g, 10.92 mmol) in Et_3N/CH_2Cl_2 (2:1; 21 mL) was stirred at room temp. for 72 h. After the reaction was complete, the mixture was concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 98:2, v/v; 96:4, v/v; 94:6, v/v) to give **5c** (0.57 g, 68%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.45–8.43 (m, 1 H, NH-Phe), 8.20–8.19 (m, 1 H, NH-Gly), 8.07–7.99 (m, 2 H, CHO and NH-β-amino-Ala), 7.29–7.15 (m, 5 H, ArH-Phe), 4.50–4.42 (m, 1 H, αH-Phe), 4.31–4.29 (m, 1 H, αH-β-amino-Ala), 3.69 (d, J = 5.7 Hz, 2 H, αCH_2-Gly), 3.58 (s, 3 H, OCH_3), 3.41 (br. s, 2 H, βNH_2-β-amino-Ala), 3.33–3.08 (m, 2 H, βCH_2-β-amino-Ala), 3.03–2.88 (m, 2 H, βCH_2-Phe) ppm. HRMS: calcd. for $C_{16}H_{22}N_4O_5$ [M + H]$^+$ 351.1663; found 351.1667.

***N*-For-Gly-L-Lys(NH$_2$·TFA)-L-Phe-OMe (5d):** Following a similar procedure to that used for the synthesis of **8**, compound **4d** (265.6 mg, 0.539 mmol), thioanisole (0.063 mL, 0.539 mmol), and trifluoroacetic acid (3 mL) in CH_2Cl_2 (9 mL) gave **5d** (272.9 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

***N*-For-L-Met-L-Lys(NH$_2$·TFA)-L-Ala-OMe (5e):** Following a similar procedure to that used for the synthesis of **8**, compound **4e** (200 mg, 0.407 mmol), thioanisole (0.048 mL, 0.407 mmol), and trifluoroacetic acid (2 mL) in CH_2Cl_2 (6 mL) gave **5e** (205.5 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

***N*-For-L-Met-L-Lys(NH$_2$·TFA)-L-Lys(Cbz)-OMe (5f):** Following a similar procedure to that used for the synthesis of **8**, compound **4f** (400 mg, 0.587 mmol), thioanisole (0.07 mL, 0.587 mmol), and trifluoroacetic acid (3 mL) in CH_2Cl_2 (9 mL) gave **5f** (408.1 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

***N*-For-L-Met-L-Lys(NH$_2$·TFA)-L-Phe-OMe (5g):** Following a similar procedure to that used for the synthesis of **8**, compound **4g** (220 mg, 0.388 mmol), thioanisole (0.046 mL, 0.388 mmol), and trifluoroacetic acid (1 mL) in CH_2Cl_2 (4 mL) gave **5g** (225.4 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

Boc-L-Ala-L-Lys-L-Ala-OMe (13): Following a similar procedure to that used for the synthesis of **2a**, compound **12** (1.10 g, 2.05 mmol) was hydrogenated in the presence of Pd/C (10%; Degussa; 0.11 g, 10% w/w) in EtOH (40 mL) to give **13** (0.82 g, quantitative) as a colourless foam, which was used directly in the next step without any further purification. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.39 (d, J = 6.4 Hz, 1 H, NH-Ala$_{C\text{-}ter}$), 7.73 (d, J = 8.0 Hz, 1 H, NH-Lys), 6.96 (d, J = 7.4 Hz, 1 H, NH-Ala$_{N\text{-}ter}$), 4.44 (br. s, 2 H, εNH_2-Lys), 4.29–4.204 (m, 2 H, αH-Lys and αH-Ala$_{C\text{-}ter}$), 4.00–3.92 (m, 1 H, αH-Ala$_{N\text{-}ter}$), 3.60 (s, 3 H, OCH_3), 2.57 (t, J = 6.9 Hz, 2 H, εCH_2-Lys), 1.69–1.45 (m, 2 H, βCH_2-Lys), 1.45–1.36 (m, 11 H, δCH_2-Lys and CH_3-*t*Bu), 1.28–1.21 (m, 5 H, CH_3-Ala$_{C\text{-}ter}$ and γCH_2-Lys), 1.15 (d, J = 7.1 Hz, 3 H, CH_3-Ala$_{N\text{-}ter}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.9 (CO-Ala$_{C\text{-}ter}$), 172.4 (CO-Ala$_{N\text{-}ter}$), 171.4 (CO-Lys), 155.0 (OCONH), 78.1 (1C of *t*Bu), 51.8 (αC-Lys and OCH_3), 49.7 (αC-Ala$_{N\text{-}ter}$), 47.5 (αC-Ala$_{C\text{-}ter}$), 40.4 (εC-Lys), 32.1 (βC-Lys), 30.7 (δC-Lys), 28.1 (CH_3-*t*Bu), 22.1 (γC-Lys), 18.0 (CH_3-Ala$_{N\text{-}ter}$), 16.8 (CH_3-Ala$_{C\text{-}ter}$) ppm. HRMS: calcd. for $C_{18}H_{34}N_4O_6$ [M + H]$^+$ 403.2551; found 403.2543.

***N*-For-L-Met-L-Lys(Boc)-L-Ala-L-Ala-OH (6a):** Following Method A, tetrapeptide **6a** was obtained as a white solid (0.99 g, 86%) in one pot without filtration of the DCU, starting from **4i** (1.0 g, 2.10 mmol), *N*-hydroxysuccinimide (0.29 g, 2.52 mmol), and DCC (0.56 g, 2.73 mmol) in THF (18 mL), and H-L-Ala-OH (0.21 g, 2.31 mmol) and $NaHCO_3$ (0.70 g, 8.39 mmol) in 1:1 THF/H_2O (40 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.55 (br. s, 1 H, CO_2H), 8.28 (d, J = 8.0 Hz, 1 H, αNH-Met), 8.10–8.03 (m, 2 H, αNH-Lys and αNH-Ala$_{N\text{-}ter}$), 8.02 (s, 1 H, CHO), 7.94 (d, J = 7.3 Hz, 1 H, αNH- Ala$_{C\text{-}ter}$), 6.73 (t, J = 5.3 Hz, 1 H, εNH-Lys), 4.47–4.40 (m, 1 H, αH-Met), 4.31–4.27 (m, 1 H, αH-Ala$_{C\text{-}ter}$), 4.24–4.16 (m, 2 H, αH-Lys and αH-Ala$_{N\text{-}ter}$), 2.89–2.84 (m, 2 H, εCH_2-Lys), 2.43 (t, J = 7.8 Hz, 2 H, γCH_2-Met), 2.03 (s, 3 H, SCH_3), 1.95–1.73 (m, 2 H, βCH_2-Met), 1.68–1.44 (m, 2 H, βCH_2-Lys), 1.40–1.33 (m, 11 H, *t*Bu and δCH_2-Lys), 1.32–1.17 (m, 8 H, 2 CH_3-Ala and γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 173.9 (CO-Ala$_{C\text{-}ter}$), 171.8 (CO-Lys), 171.0 (CO-Ala$_{N\text{-}ter}$), 170.6 (CO-Met), 160.9 (CHO), 155.5 (OCONH), 77.3 (1C of *t*Bu), 52.5 (αC-Lys), 50.4 (αC-Met), 47.7 (αC-Ala$_{C\text{-}ter}$), 47.4 (αC-Ala$_{N\text{-}ter}$), 40.2 (εC-Lys), 32.2 (βC-Met), 31.5 (βC-Lys), 29.3 (γC-Met), 29.2 (δC-Lys), 28.3 (CH_3-*t*Bu), 22.7 (γC-Lys), 18.1 (CH_3-Ala$_{C\text{-}ter}$), 17.2 (CH_3-Ala$_{N\text{-}ter}$), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{23}H_{41}N_5O_8S$ [M + Na]$^+$ 570.2568; found 570.2571.

***N*-For-L-Met-L-Lys(Boc)-L-Ala-L-Phe-OMe (6b):** Following Method B, tetrapeptide **6b** was obtained as a white solid (0.55 g, 82%) in one pot without filtration of the DCU, starting from **4i** (0.5 g, 1.05 mmol), *N*-hydroxysuccinimide (0.15 g, 1.26 mmol), and DCC (0.28 g, 1.36 mmol) in THF (10 mL), and H-L-Phe-OMe·HCl (0.25 g, 1.15 mmol) and $NaHCO_3$ (0.26 g, 3.15 mmol) in 1:1 THF/H_2O (30 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.29–8.24 (m, 2 H, αN*H*-Met and αN*H*-Phe), 8.05–8.02 (m, 2 H, C*H*O and αN*H*-Lys), 7.90 (d, J = 7.4 Hz, 1 H, αN*H*-Ala), 7.30–7.19 (m, 5 H, Ar-*H* Phe), 6.73 (t, J = 5.7 Hz, 1 H, εN*H*-Lys), 4.49–4.44 (m, 1 H, α*H*-Met), 4.32–4.27 (m, 1 H, α*H*-Ala), 4.25–4.19 (m, 1 H, α*H*-Lys), 3.57 (s, 3 H, OCH_3), 3.05–2.94 (m, 2 H, βCH_2-Phe), 2.92–2.82 (m, 2 H, εCH_2-Lys), 2.43 (t, J = 7.8 Hz, 2 H, γCH_2-Met), 2.02 (s, 3 H, -SCH_3), 1.95–1.73 (m, 2 H, βCH_2-Met), 1.65–1.44 (m, 2 H, βCH_2-Lys), 1.40–1.24 (m, 11 H, *t*Bu and δCH_2-Lys), 1.24–1.13 (m, 5 H, CH_3-Ala and γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO): δ = 172.2 (CO-Ala), 171.7 (CO-Phe), 171.0 (CO-Lys), 170.6 (CO-Met), 160.9 (CHO), 155.5 (OCONH), 136.9 (1C of Phe), 129.0 (Ar-C), 128.2 (Ar-C), 126.5 (Ar-C), 77.3 (1C of *t*Bu), 53.5 (αC-Phe), 52.4 (αC-Lys), 51.8 (OCH_3), 50.4 (αC-Met), 47.8 (αC-Ala), 40.2 (εC-Lys), 36.6 (βC-Phe), 32.2 (βC-Met), 31.5 (βC-Lys), 29.3 (γC-Met), 29.2 (δC-Lys), 28.2 (CH_3-*t*Bu), 22.7 (γC-Lys), 18.2 (CH_3-Ala), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{30}H_{47}N_5O_8S$ [M + Na]$^+$ 660.3038; found 660.3049.

***N*-For-L-Met-L-Lys(NH$_2$·TFA)-L-Ala-L-Phe-OMe (9):** Following a similar procedure to that used for the synthesis of **8**, compound **6b** (248 mg, 0.388 mmol), thioanisole (0.046 mL, 0.388 mmol), and trifluoroacetic acid (1 mL) in CH_2Cl_2 (4 mL) gave **9** (253 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

***N*-For-L-Met-L-Lys(Boc)-L-Ala-L-Ala-L-Phe-OMe (7):** Following Method B, pentapeptide **7** was obtained as a white solid (0.87 g, 81%) in two steps, starting from **6a** (0.83 g, 1.51 mmol), *N*-hydroxysuccinimide (0.21 g, 1.82 mmol), and DCC (0.41 g, 1.97 mmol) in THF (20 mL), and H-L-Phe-OMe·HCl (0.36 g, 1.67 mmol) and $NaHCO_3$ (0.38 g, 4.54 mmol) in 1:1 THF/H_2O (38 mL). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.28 (d, J = 8.3 Hz, 1 H, αN*H*-Met), 8.23 (d, J = 7.6 Hz, 1 H, αN*H*-Phe), 8.06 (d, J = 7.7 Hz, 1 H, αN*H*-Lys), 8.02 (s, 1 H, C*H*O), 7.96 (d, J = 7.2 Hz, 1 H, αN*H*-Ala), 7.85 (d, J = 7.7 Hz, 1 H, αN*H*-Ala), 7.30–7.19 (m, 5 H, Ar-*H* Phe), 6.73 (t, J = 5.4 Hz, 1 H, εN*H*-Lys), 4.49–4.40 (m, 2 H, α*H*-Met and α*H*-Phe), 4.30–4.20 (unresolved m, 3 H, 2 α*H*-Ala and α*H*-Lys), 3.58 (s, 3 H, OCH_3), 3.06–2.93 (m, 2 H, βCH_2-Phe), 2.90–2.84 (m, 2 H, εCH_2-Lys), 2.43 (t, J = 7.7 Hz, 2 H, γCH_2-Met), 2.03 (s, 3 H, SCH_3), 1.95–1.73 (m, 2 H, βCH_2-Met), 1.72–1.47 (m, 2 H, βCH_2-Lys), 1.40–1.31 (m, 11 H, *t*Bu and δCH_2-Lys), 1.24–1.12 (m, 8 H, 2 CH_3-Ala and γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO): δ = 172.2 (CO-Ala$_{C\text{-ter}}$), 171.7 (CO-Phe), 171.5 (CO-Ala$_{N\text{-ter}}$), 171.2 (CO-Lys), 170.6 (CO-Met), 161.0 (CHO), 155.5 (OCONH), 137.0 (1C of Phe), 129.0 (Ar-C), 128.2 (Ar-C), 126.5 (Ar-C), 77.3 (1C of *t*Bu), 53.5 (αC-Phe), 52.5 (αC-Lys), 51.8 (OCH_3), 50.4 (αC-Met), 47.9 (αC-Ala), 47.9 (αC- Ala$_{C\text{-ter}}$), 47.7 (αC- Ala$_{N\text{-ter}}$), 40.2 (εC-Lys), 36.6 (βC-Phe), 32.2 (βC-Met), 31.5 (βC-Lys), 29.3 (γC-Met), 29.2 (δC-Lys), 28.3 (CH_3-*t*Bu), 22.7 (γC-Lys), 18.2 (CH_3-Ala), 17.9 (CH_3-Ala), 14.6 (SCH_3) ppm. HRMS: calcd. for $C_{33}H_{52}N_6O_9S$ [M – H]$^-$ 707.3443; found 707.3451.

***N*-For-L-Met-L-Lys(NH$_2$·TFA)-L-Ala-L-Ala-L-Phe-OMe (10):** Following a similar procedure to that used for the synthesis of **8**, compound **7** (275 mg, 0.388 mmol), thioanisole (0.046 mL, 0.388 mmol), and trifluoroacetic acid (1 mL) in CH_2Cl_2 (4 mL) gave **10** (281 mg, quantitative) as a colourless foam, which was used directly in the next step without any further purification.

5′-*O*-(4-Monomethoxytrityl)-2′-deoxythymidine (14):[23] Triethylamine (3.61 mL, 25.8 mmol) was added to a stirred solution of thymidine (2.50 g, 10.32 mmol) in dry DMF (26 mL). DMAP (0.10 g, 0.825 mmol) and 4-monomethoxytrityl chloride (6.37 g, 20.64 mmol) were then added, and the resulting mixture was stirred at room temp. for 4 h. The reaction mixture was quenched with water (260 mL), and the aqueous layer was extracted with ethyl acetate (3× 150 mL). The combined organic extracts were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 2:1, v/v; 1:1, v/v; 1:2, v/v) to give **14** (5.14 g, 97%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.79 (br. s, 1 H, N*H*), 7.57 (d, J = 1.1 Hz, 1 H, 6-H), 7.42–7.39 (m, 4 H, Ar-*H*), 7.33–7.22 (m, 8 H, Ar-*H*), 6.86–6.83 (m, 2 H, Ar-*H*), 6.42 (dd, J = 7.6, 6.1 Hz, 1 H, 1′-H), 4.59–4.57 (m, 1 H, 3′-H), 4.08–4.05 (m, 1 H, 4′-H), 3.79 (s, 3 H, OCH_3), 3.49–3.35 (m, 2 H, 5′-H and 5′′-H), 2.44–2.29 (m, 2 H, 2′-H and 2′′-H), 1.47 (d, J = 1.1 Hz, 3 H, CH_3) ppm. HRMS: calcd. for $C_{30}H_{30}N_2O_6$ [M + Na]$^+$ 537.1996; found 537.1998.

5′-*O*-(4-Monomethoxytrityl)-3′-*O*-(4-nitrophenyl carbonate)-2′-deoxythymidine (15): Compound **14** (1.00 g, 1.95 mmol) was coevaporated with dry pyridine (2× 5 mL), then it was redissolved in dry pyridine (5 mL). A suspension of 4-nitrophenyl chloroformate (0.432 g, 2.14 mmol) in a mixture of CH_2Cl_2/pyridine (70:1; 5 mL) was slowly added to this solution at 0 °C. The reaction mixture was then stirred overnight at room temp. The volatiles were removed under reduced pressure, and the resulting residue was redissolved in CH_2Cl_2 (4 mL). This solution was slowly added into vigorously stirring diethyl ether (30 mL). The resulting precipitate was filtered off, and the filtrate was poured into stirring hexane (100 mL) to give a white precipitate, which was filtered and dried to give **15** (0.87 g, 66%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.81 (br. s, 1 H, N*H*), 8.31–8.27 (m, 2 H, Ar-*H*), 7.61 (s, 1 H, 6-H), 7.39–7.26 (m, 14 H, Ar-*H*), 6.87–6.84 (m, 2 H, Ar-*H*), 6.39 (dd, J = 8.8, 5.6 Hz, 1 H, 1′-H), 5.51–5.45 (m, 1 H, 3′-H), 4.32 (br. s, 1 H, 4′-H), 3.79 (s, 3 H, OCH_3), 3.60–3.47 (m, 2 H, 5′-H and 5′′-H), 2.79–2.47 (m, 2 H, 2′-H and 2′′-H), 1.43 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.6, 159.1, 155.2, 152.0, 150.5, 145.7, 143.7, 135.2, 134.6, 130.5, 128.5, 128.4, 128.3, 127.6, 125.5, 122.2, 121.8, 113.5 112.0, 84.4, 83.6, 80.5, 63.9, 55.4, 38.0, 11.8 ppm. HRMS: calcd. for $C_{37}H_{33}N_3O_{10}$ [M + Na]$^+$ 702.2058; found 702.2081.

5′-*O*-(4-Monomethoxytrityl)-3′-*O*-[*N*-For-Gly-L-Lys(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine (16): Compound **15** (132 mg, 0.195 mmol) in dry CH_2Cl_2 was added to a stirred solution of tripeptide **5d** (110 mg, 0.217 mmol) and Et_3N (0.105 mL, 0.868 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C, and the stirring was continued at room temp. for 16 h. The reaction mixture was then diluted with CH_2Cl_2 and washed twice with $NaHCO_3$ (10% aq.). The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/EtOH, 99:1, v/v; 97:3, v/v; 94:6, v/v) to give **16** (160 mg, 79%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.39 (br. s, 1 H, N*H*), 8.40 (d, J = 7.4 Hz, 1 H, N*H*-Phe), 8.22 (t, J = 5.4 Hz, 1 H, N*H*-Gly), 8.06 (s, 1 H, C*H*O), 7.99 (d, J = 8.2 Hz, 1 H, N*H*-Lys), 7.53 (s, 1 H, 6-H), 7.53–7.19 (m, 18 H, Ar-*H* and εN*H*-Lys), 6.23 (dd, J = 7.8, 6.5 Hz, 1 H, 1′-H), 5.24–5.23 (m, 1 H, 3′-H), 4.48–4.41 (m, 1 H, αH-Phe), 4.31–4.25 (m, 1 H, αH-Lys), 4.05 (br. s, 1 H, 4′-H), 3.75 (s, 2 H, CH_2-Gly), 3.56 (s, 3 H, OCH_3), 3.39–3.20 (m, 2 H, 5′-H and 5′′-H), 2.99–2.94 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.47–2.25 (m, 1 H, 2′-H and 2′′-H), 1.77 (s, 3 H, CH_3-Thy), 1.62–1.55 (m, 2 H, βCH_2-Lys), 1.54–1.45 (m, 5 H, δCH_2-Lys and CH_3-

Thy), 1.37–1.24 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO): δ = 171.7, 171.6, 168.1, 163.6, 161.4, 158.3, 155.2, 150.4, 144.1, 143.8, 137.1, 135.4, 134.6, 130.1, 129.0, 128.2, 127.9, 127.1, 126.5, 113.3, 109.9, 83.6, 83.2, 74.2, 63.9, 55.1, 63.6, 52.1, 51.7, 40.4, 36.8, 36.5, 31.9, 29.1, 22.4, 11.6 ppm. HRMS: calcd. for $C_{50}H_{56}N_6O_{12}$ [M + Na]$^+$ 955.3848; found 955.3813.

5′-Hydroxy-3′-*O*-[*N*-For-Gly-L-Lys(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine (17): A solution of **16** (147 mg, 0.157 mmol) in AcOH (80%; 2 mL) was stirred at room temp. for 3.5 h. The volatiles were then removed under reduced pressure, and the residue was coevaporated twice with toluene. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 100:0, v/v; 98:2, v/v; 96:5, v/v) to give **17** (85 mg, 82%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.32 (br. s, 1 H, N*H*), 8.38 (d, *J* = 7.3 Hz, 1 H, N*H*-Phe), 8.19 (t, *J* = 5.1 Hz, 1 H, N*H*-Gly), 8.06 (s, 1 H, C*H*O), 7.96 (d, *J* = 7.9 Hz, 1 H, N*H*-Lys), 7.73 (s, 1 H, 6-H), 7.31–7.19 (m, 5 H, Ar-*H*), 6.17 (dd, *J* = 8.5, 5.9 Hz, 1 H, 1′-H), 5.19 (t, *J* = 4.7 Hz, 1 H, εN*H*-Lys), 5.11–5.09 (m, 1 H, 3′-H), 4.48–4.40 (m, 1 H, αH-Phe), 4.31–4.23 (m, 1 H, αH-Lys), 3.93 (br. s, 1 H, 4′-H), 3.75 (d, *J* = 5.6 Hz, 2 H, αCH_2-Gly), 3.63–3.60 (m, 2 H, 5′-H and 5′′-H), 3.57 (s, 3 H, OCH_3), 3.05–2.89 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.33–2.17 (m, 2 H, 2′-H and 2′′-H), 1.78 (s, 3 H, CH_3-Thy), 1.59–1.47 (m, 2 H, βCH_2-Lys), 1.46–1.34 (m, 2 H, δCH_2-Lys), 1.28–1.15 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.8, 171.6, 168.1, 163.7, 161.4, 155.4, 150.5, 137.1, 135.8, 129.1, 128.3, 126.6, 109.7, 85.5, 83.7, 74.6, 61.5, 40.4, 36.8, 36.5, 31.9, 30.7, 29.1, 22.4, 12.3 ppm. HRMS: calcd. for $C_{30}H_{40}N_6O_{11}$ [M + H]$^+$ 661.2827; found 661.2828.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-Gly-L-Lys(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine (18): Tetrazole (0.45 M in MeCN; 3.53 mL, 1.59 mmol) and then dibenzyldiisopropyl phosphoramidite (0.24 mL, 0.697 mmol) were added to a stirred suspension of **17** (210 mg, 0.317 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C, and the reaction mixture was stirred at room temp. for 12 h. The mixture was cooled to –40 °C, then H_2O_2 (35%; 0.14 mL, 1.59 mmol) was slowly added, and the resulting mixture was stirred first at 0 °C for 1 h and then at room temp. for 1 h. The mixture was cooled and quenched with $NaHSO_3$ (10% w/v aq.; 6 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic extracts were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 96:4, v/v) to give **18** (230 mg, 79%) as a colourless foam.

5′-*O*-(4-Monomethoxytrityl)-3′-*O*-benzoyl-2′-deoxythymidine (20): Benzoyl chloride (0.80 mL, 6.91 mmol) was slowly added to a stirred solution of **14** (3.09 g, 6.01 mmol) in pyridine (25 mL) at 0 °C, and the resulting mixture was stirred for 3 h at 0 °C. The reaction mixture was then diluted with CH_2Cl_2 (200 mL), and the mixture was washed with saturated aq. $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4, filtered, evaporated and coevaporated with toluene. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 3:1, v/v; 2:1, v/v; 1:1, v/v) to give **20** (3.53 g, 95%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.56 (br. s, 1 H, N*H*), 8.06–8.03 (m, 2 H, Ar-*H*), 7.66 (s, 1 H, 6-H), 7.60–7.25 (m, 15 H, Ar-*H*), 6.87–6.84 (m, 2 H, Ar-*H*), 6.56 (dd, *J* = 8.7, 5.7 Hz, 1 H, 1′-H), 5.74–5.72 (m, 1 H, 3′-H), 4.30–4.29 (m, 1 H, 4′-H), 3.78 (s, 3 H, OCH_3), 3.61–3.51 (m, 2 H, 5′-H and 5′′-H), 2.69–2.51 (m, 2 H, 2′-H and 2′′-H), 1.43 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.0, 164.0, 158.9, 150.8, 143.9, 143.8, 135.4, 134.8, 133.6, 130.5, 129.8, 129.4, 128.6, 128.5, 128.4, 128.1, 127.4, 113.4, 111.8, 87.5, 84.5, 84.2, 75.9, 63.9, 55.3, 38.2, 11.8 ppm. HRMS: calcd. for $C_{37}H_{34}N_2O_7$ [M + Na]$^+$ 641.2258; found 641.2257.

5′-Hydroxy-3′-*O*-benzoyl-2′-deoxythymidine (21):[52] A solution of **20** (3.35 g, 5.41 mmol) in AcOH (80%; 40 mL) was stirred at room temp. for 3.5 h. The volatiles were then removed under reduced pressure, and the residue was coevaporated twice with toluene. The residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 100:0, v/v; 98:2, v/v; 96:4, v/v) to give **21** (1.7 g, 91%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.35 (br. s, 1 H, N*H*), 8.03–8.00 (m, 2 H, *o*-H of Ph), 7.79 (d, *J* = 1.1 Hz, 1 H, 6-H), 7.71–7.66 (m, 1 H, *p*-H of Ph), 7.58–7.53 (m, 2 H, *m*-H of Ph), 6.30 (app t, *J* = 7.1 Hz, 1 H, 1′-H), 5.49–5.48 (m, 1 H, 3′-H), 5.26 (br. s, 1 H, O*H*), 4.18–4.15 (m, 1 H, 4′-H), 3.71 (br. s, 2 H, 5′-H and 5′′-H), 2.43–2.39 (m, 2 H, 2′-H and 2′′-H), 1.80 (d, *J* = 1.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 165.2 (COPh), 163.7 (C-4), 150.5 (C-2), 135.9 (C-6), 133.6 (*p*-C of Ph), 129.4 (1C of Ph), 129.3 (*o*-C of Ph), 128.8 (*m*-C of Ph), 109.8 (C-5), 84.6 (C-1′), 83.8 (C-4′), 75.7 (C-3′), 61.4 (C-5′), 36.7 (C-2′), 12.3 (CH$_3$) ppm. HRMS: calcd. for $C_{37}H_{34}N_2O_7$ [M + H]$^+$ 347.1237; found 347.1238.

5′-*O*-(Dibenzylphosphate)-3′-*O*-benzoyl-2′-deoxythymidine (22): Tetrazole (0.45 M in MeCN; 51.33 mL, 23.10 mmol) and then dibenzyldiisopropyl phosphoramidite (3.47 mL, 10.16 mmol) were added to a stirred suspension of **21** (1.6 g, 4.62 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C, and the reaction mixture was stirred at room temp. for 12 h. The mixture was cooled to –40 °C, H_2O_2 (35%; 1.97 mL, 23.10 mmol) was slowly added, and the resulting mixture was stirred at 0 °C for 1 h and for an additional 1 h at room temp. Then the reaction mixture was again cooled to 0 °C and finally quenched with $NaHSO_3$ (10% w/v aq.; 30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic extracts were dried with Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 3:1, v/v; 2:1, v/v; 1:1, v/v) to give **22** (2.59 g, 92%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.38 (br. s, 1 H, N*H*), 8.05–8.02 (m, 2 H, Ar-*H*), 7.63–7.58 (m, 1 H, Ar-*H*), 7.49–7.44 (m, 3 H, 6-H and Ar-H), 7.35 (br. s, 10 H, Ar-*H*), 6.46 (dd, *J* = 9.0, 5.4 Hz, 1 H, 1′-H), 5.37–5.35 (m, 1 H, 3′-H), 5.15–5.03 (m, 4 H, OCH_2Ph), 4.38–4.34 (m, 1 H, 4′-H), 4.25 (br. s, 2 H, 5′-H and 5′′-H), 2.48–2.42 (m, 2 H, 2′-H and 2′′-H), 1.86 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.0 (COPh), 163.8 (C-4), 150.7 (C-2), 135.5 (d, $^3J_{C,P}$ = 6.0 Hz, 1C of OCH$_2$Ph), 135.0 (C-6), 133.7 (Ar-C), 129.8 (Ar-C), 129.1 (Ar-C), 129.0 (Ar-C), 128.8 (Ar-C), 128.6 (Ar-C), 128.2 (Ar-C), 111.9 (C-5), 84.6 (C-1′), 83.0 (d, $^3J_{C,P}$ = 8.3 Hz, C-4′), 75.3 (C-3′), 69.9 (app t, $^2J_{C,P}$ = 5.3 Hz, OCH$_2$), 67.3 (d, $^2J_{C,P}$ = 5.7 Hz, C-5′), 37.3 (C-2′), 12.4 (CH$_3$) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.6 ppm. HRMS: calcd. for $C_{37}H_{34}N_2O_7$ [M + H]$^+$ 607.1840; found 607.1840.

5′-*O*-(Dibenzylphosphate)-2′-deoxythymidine (23): A solution of **22** (2.52 g, 4.15 mmol) in NH_3 (7 N in MeOH; 30 mL) was stirred at 0 °C for 3 h and then at room temp. for 24 h. The volatiles were removed under reduced pressure, and the crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 97:3, v/v) to give **23** (1.98 g, 95%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.41 (br. s, 1 H, N*H*), 7.34–7.33 (m, 11 H, 6-H and Ar-*H*), 6.29 (app t, *J* = 6.7 Hz, 1 H, 1′-H), 5.11–4.99 (m, 4 H, OCH_2Ph), 4.37–4.33 (m, 1 H, 3′-H), 4.22–4.14 (m, 2 H, 5′-H and 5′′-H), 4.04–4.00 (m, 2 H, 4′-H), 2.35–2.27 (m, 1 H, 2′-H), 2.03–1.96 (m, 1 H, 2′′-H), 1.81 (d, *J* = 1.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0 (C-4), 150.7 (C-2), 149.8 (1C of PhNO$_2$), 145.36 (*p*-C of PhNO$_2$),

135.7 (C-6), 135.4 (d, $^3J_{C,P}$ = 7.3 Hz, 1C of OCH_2Ph), 129.1 (Ar-C), 128.9 (Ar-C), 128.2 (Ar-C), 111.5 (C-5), 85.0 (C-1′), 84.8 (d, $^3J_{C,P}$ = 7.5 Hz, C-4′), 71.1 (C-3′), 70.1–70.0 (2 d, $^2J_{C,P}$ = 5.6 Hz, 2 OCH_2Ph), 66.9 (d, $^2J_{C,P}$ = 5.7 Hz, C-5′), 40.2 (C-2′), 12.5 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.5 ppm. HRMS: calcd. for $C_{24}H_{27}N_2O_8P$ [M + H]$^+$ 503.1578; found 503.1580.

5′-*O*-(Dibenzylphosphate)-3′-*O*-(4-nitrophenyl Carbonate)-2′-deoxythymidine (24): Compound **23** (1.12 g, 2.23 mmol) was coevaporated with dry pyridine (2× 5 mL), then it was redissolved in dry pyridine (7 mL), and a suspension of 4-nitrophenyl chloroformate (0.539 g, 2.67 mmol) in a mixture of CH_2Cl_2/pyridine (70:1; 7.1 mL) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, and at room temp. for 24 h. The volatiles were removed under reduced pressure, the residue was redissolved in CH_2Cl_2 (150 mL), and this solution was washed with saturated aq. $NaHCO_3$ (2× 70 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 2:1, v/v; 1:1, v/v; 1:3, v/v) to give **24** (0.99 g, 67%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.43 (br. s, 1 H, N*H*), 8.32–8.29 (m, 2 H, Ar-*H*), 7.41–7.26 (m, 13 H, 6-H and Ar-*H*), 6.39 (dd, *J* = 9.3, 5.3 Hz, 1 H, 1′-H), 5.17–5.15 (m, 1 H, 3′-H), 5.12–5.00 (m, 4 H, OCH_2Ph), 4.28–4.27 (m, 1 H, 4′-H), 4.26–4.20 (m, 2 H, 5′-H and 5′′-H), 2.48–2.41 (m, 1 H, 2′-H), 2.04–1.93 (m, 1 H, 2′′-H), 1.86 (d, *J* = 1.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.9 (C-4), 154.7 ($COPhNO_2$), 151.5 (C-2), 149.8 (1C of $PhNO_2$), 145.36 (*p*-C of $PhNO_2$), 135.0 (d, $^3J_{C,P}$ = 5.8 Hz, 1C of OCH_2Ph), 134.4 (C-6), 128.7 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 127.8 (Ar-C), 125.1 (Ar-C), 121.3 (Ar-C), 111.6 (C-5), 84.1 (C-1′), 81.9 (d, $^3J_{C,P}$ = 8.2 Hz, C-4′), 79.1 (C-3′), 69.6 (app t, $^2J_{C,P}$ = 4.9 Hz, OCH_2Ph), 66.6 (d, $^2J_{C,P}$ = 5.5 Hz, C-5′), 36.7 (C-2′), 12.0 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.5 ppm. HRMS: calcd. for $C_{31}H_{30}N_3O_{12}P$ [M + H]$^+$ 668.1640; found 668.1638.

General Procedure for the Synthesis of 3′-Carbamoyl Peptide Conjugates of dTMP (18 and 25–32): A solution of compound **24** in dry CH_2Cl_2 was added to a stirred solution of peptide-NH_2·TFA salt (1 equiv.) and Et_3N (4 equiv.) in dry CH_2Cl_2 at 0 °C, and the stirring was continued at room temp. for 24 h. The reaction mixture was then diluted with CH_2Cl_2 and washed twice with $NaHCO_3$ (20% aq.). The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 96:4, v/v) to give the desired 3′-carbamoyl PNCs.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-Gly-L-β-amino-Ala-(β-carbamate)-L-Phe-OMe]-2′-deoxythymidine (25): Compound **25** was prepared according to the general procedure starting from **24** (240 mg, 0.365 mmol), tripeptide **5c** (179 mg, 0.510 mmol), and Et_3N (0.05 mL, 0.539 mmol) in dry CH_2Cl_2 (15 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:5, v/v) to give conjugate **25** (259 mg, 82%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.93 (br. s, 1 H, N*H*-Thy), 8.18 (s, 1 H, C*H*O), 7.65 (d, *J* = 7.2 Hz, 1 H, N*H*-Phe), 7.65 (d, *J* = 7.2 Hz, 1 H, N*H*-β-amino-Ala), 7.39 (s, 1 H, 6-H), 7.32 (br. s, 10 H, Ar-*H* of OBn), 7.26–7.09 (m, 5 H, Ar-*H* of Phe), 6.39 (t, *J* = 6.3 Hz, 1 H, N*H*-Gly), 6.25 (dd, *J* = 8.3, 5.2 Hz, 1 H, 1′-H), 5.07–4.98 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.83–4.76 (m, 1 H, α*H*-Phe), 4.75–4.68 (m, 1 H, α*H*-β-amino-Ala), 4.28–4.17 (m, 2 H, 5′-H and 5′′-H), 4.14 (br. s, 1 H, 4′-H), 4.04–3.88 (m, 2 H, CH_2-Gly), 3.67 (s, 3 H, OCH_3), 3.58–3.33 (m, 2 H, βCH_2-β-amino-Ala), 3.16–2.99 (m, 2 H, βCH_2-Phe), 2.38–2.14 (m, 2 H, 2′-H and 2′′-H), 1.80 (s, 3 H, CH_3-Thy) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0 (CO-Phe), 169.7 (CO-β-amino-Ala), 169.4 (CO-Gly), 164.0 (C-4), 162.4 (CHO), 156.5 (OCONH), 151.1 (C-2), 136.1 (C-6), 135.5 (d, $^3J_{C,P}$ = 5.5 Hz, 1C of OCH_2Ph), 135.1 (1C Ph-Phe), 129.3 (Ar-C), 129.0 (Ar-C), 128.9 (Ar-C), 128.6 (Ar-C), 128.2 (Ar-C), 127.2 (Ar-C), 112.0 (C-5), 84.6 (C-1′), 82.8 (d, $^3J_{C,P}$ = 7.7 Hz, C-4′), 75.5 (C-3′), 69.9 (app t, $^2J_{C,P}$ = 5.0 Hz, 2 OCH_2Ph), 67.4 (d, $^2J_{C,P}$ = 4.9 Hz, C-5′), 53.9 (αC-Phe), 53.6 (αC-β-amino-Ala), 52.6 (OCH_3), 43.3 (βC-β-amino-Ala), 41.7 (αC-Gly), 37.8 (C-2′), 37.2 (βC-Phe), 12.5 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.7 ppm. HRMS: calcd. for $C_{41}H_{47}N_6O_{14}P$ [M + H]$^+$ 879.2960; found 879.2966.

5′-*O*-(Dibenzylphosphate)-*3′*-*O*-[*N*-For-Gly-L-Lys(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine (18): Compound **18** was prepared according to the general procedure starting from **24** (300 mg, 0.449 mmol), tripeptide **5d** (273 mg, 0.539 mmol), and Et_3N (0.25 mL, 1.80 mmol) in dry CH_2Cl_2 (15 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:2, v/v; 92:4, v/v) to give conjugate **18** (322 mg, 78%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_4$]-methanol): δ = 8.15 (s, 1 H, C*H*O), 7.46 (s, 1 H, 6-H), 7.36 (br. s, 10 H, Ar-*H* of OBn), 7.29–7.17 (m, 5 H, Ar-*H* of Phe), 6.23 (dd, *J* = 8.6, 5.8 Hz, 1 H, 1′-H), 5.12–5.08 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.67–4.63 (m, 1 H, α*H*-Phe), 4.38–4.33 (m, 1 H, α*H*-Lys), 4.30–4.26 (m, 2 H, 5′-H and 5′′-H), 4.17–4.16 (m, 1 H, 4′-H), 3.90 (s, 2 H, CH_2-Gly), 3.67 (s, 3 H, OCH_3), 3.16–2.96 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.33–2.27 (m, 1 H, 2′-H), 2.12–2.02 (m, 1 H, 2′′-H), 1.77 (s, 3 H, CH_3-Thy), 1.71–1.58 (m, 2 H, βCH_2-Lys), 1.51–1.47 (m, 2 H, δCH_2-Lys), 1.33–1.29 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_4$]methanol): δ = 173.9 (CO-Phe), 173.3 (CO-Lys), 170.7 (CO-Gly), 166.1 (C-4), 164.2 (CHO), 157.8 (OCONH), 152.2 (C-2), 138.1 (1C Ph-Phe), 137.1 (C-6), 137.0 (d, $^3J_{C,P}$ = 6.1 Hz, 1C of OCH_2Ph), 130.3 (Ar-C), 129.9 (Ar-C), 129.8 (Ar-C), 129.5 (Ar-C), 129.2 (Ar-C), 127.9 (Ar-C), 112.1 (C-5), 86.2 (C-1′), 84.4 (d, $^3J_{C,P}$ = 7.7 Hz, C-4′), 75.7 (C-3′), 71.2–71.1 (2 d, $^2J_{C,P}$ = 5.7 Hz, 2 OCH_2Ph), 68.7 (d, $^2J_{C,P}$ = 5.8 Hz, C-5′), 55.2 (αC-Phe), 54.4 (αC-Lys), 52.7 (OCH_3), 42.0 (αC-Gly), 41.5 (εC-Lys), 38.3 (C-2′), 38.1 (βC-Phe), 32.8 (βC-Lys), 30.3 (δC-Lys), 23.8 (γC-Lys), 12.5 (CH_3) ppm. ^{31}P NMR (121 MHz, [D$_4$]methanol): δ = –1.2 ppm. HRMS: calcd. for $C_{44}H_{53}N_6O_{14}P$ [M – H]$^-$ 919.3284; found 919.3287.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine (26): Compound **26** was prepared according to the general procedure starting from **24** (246.2 mg, 0.367 mmol), tripeptide **5g** (225.4 mg, 0.3882 mmol), and Et_3N (0.22 mL, 1.55 mmol) in dry CH_2Cl_2 (20 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:5, v/v) to give conjugate **26** (330 mg, 85%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]-DMSO): δ = 11.36 (br. s, 1 H, N*H*-Thy), 8.33 (d, *J* = 7.5 Hz, 1 H, N*H*-Phe), 8.27 (d, *J* = 8.4 Hz, 1 H, N*H*-Met), 8.01–8.00 (m, 2 H, C*H*O and αN*H*-Lys), 7.50 (s, 1 H, 6-H), 7.36–7.34 (m, 11 H, Ar-*H* of OBn and εN*H*-Lys), 7.26–7.19 (m, 5 H, Ar-*H* of Phe), 6.19 (app t, *J* = 7.3 Hz, 1 H, 1′-H), 5.07–5.04 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.50–4.39 (m, 2 H, α*H*-Met and α*H*-Phe), 4.26–4.20 (unresolved m, 3 H, α*H*-Lys, 5′-H, and 5′′-H), 4.10 (br. s, 1 H, 4′-H), 3.56 (s, 3 H, OCH_3), 3.05–2.89 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.39 (t, *J* = 7.8 Hz, 2 H, γCH_2-Met), 2.23–2.18 (m, 2 H, 2′-H and 2′′-H), 2.00 (s, 3 H, SCH_3), 1.91–1.72 (m, 2 H, βCH_2-Met), 1.69 (s, 3 H, CH_3-Thy), 1.63–1.45 (m, 2 H, βCH_2-Lys), 1.40–1.36 (m, 2 H, δCH_2-Lys), 1.28–1.19 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.7 (CO-Phe), 171.5 (CO-Lys), 170.4 (CO-Met), 163.5 (C-4), 160.9 (CHO), 155.2 (OCONH), 150.4 (C-

2), 137.0 (1C of Phe), 135.9 (d, $^3J_{C,P}$ = 6.8 Hz, 1C of OCH_2Ph), 135.4 (C-6), 129.0 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 128.2 (Ar-C), 127.8 (Ar-C), 126.5 (Ar-C), 110.0 (C-5), 83.9 (C-1′), 82.2 (d, $^3J_{C,P}$ = 7.4 Hz, C-4′), 73.6 (C-3′), 68.7 (2 d, $^2J_{C,P}$ = 5.5 Hz, 2 OCH_2Ph), 67.0 (d, $^2J_{C,P}$ = 5.7 Hz, C-5′), 53.4 (αC-Phe), 52.2 (αC-Lys), 51.8 (OCH_3), 50.3 (αC-Met), 40.2 (εC-Lys), 36.5 (βC-Phe), 36.0 (C-2′), 32.2 (βC-Met), 31.7 (βC-Lys), 29.3 (γC-Met), 29.0 (δC-Lys), 22.5 (γC-Lys), 14.6 (-SCH_3), 12.0 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, [D$_6$]DMSO): δ = –0.9 ppm. HRMS: calcd. for $C_{47}H_{59}N_6O_{14}PS$ [M – H]$^-$ 993.3474; found 993.3478.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Ala-OMe]-2′-deoxythymidine (27): Compound **27** was prepared according to the general procedure starting from **24** (226.6 mg, 0.339 mmol), tripeptide **5e** (205.5 mg, 0.407 mmol), and Et_3N (0.19 mL, 1.36 mmol) in dry CH_2Cl_2 (15 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:5, v/v) to give conjugate **27** (224.5 mg, 72%) as a colourless foam. ^{1}H NMR (500 MHz, [D$_6$]-DMSO): δ = 11.3 (br. s, 1 H, N*H*-Thy), 8.31 (d, *J* = 6.8 Hz, 1 H, N*H*-Ala), 8.27 (d, *J* = 8.1 Hz, 1 H, N*H*-Met), 8.03–8.01 (m, 2 H, C*H*O and αN*H*-Lys), 7.50 (s, 1 H, 6-H), 7.36–7.35 (m, 11 H, Ar-*H* of OBn and εN*H*-Lys), 6.19 (app t, *J* = 7.3 Hz, 1 H, H-1′), 5.08–5.05 (m, 5 H, H-3′ and 2 OCH_2Ph), 4.46–4.41 (m, 1 H, α*H*-Met), 4.27–4.22 (unresolved m, 4 H, α*H*-Lys, α*H*-Ala, H-5′, and H-5′′), 4.11 (br. s, 1 H, H-4′), 3.61 (s, 3 H, OCH_3), 2.99–2.95 (m, 2 H, εCH_2-Lys), 2.42 (t, *J* = 7.9 Hz, 2 H, γCH_2-Met), 2.22–2.19 (m, 2 H, H-2′ and H-2′′), 2.02 (s, 3 H, SCH_3), 1.91–1.72 (m, 2 H, βCH_2-Met), 1.69 (s, 3 H, CH_3-Thy), 1.67–1.48 (m, 2 H, βCH_2-Lys), 1.43–1.37 (m, 2 H , δ CH_2-Lys), 1.33–1.22 (m, 5 H, γCH_2-Lys and CH_3-Ala). ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 172.9 (CO-Ala), 171.4 (CO-Lys), 170.5 (CO-Met), 163.6 (C-4), 161.0 (CHO), 155.2 (OCONH), 150.4 (C-2), 135.9 (d, $^3J_{C,P}$ = 6.9 Hz, 1C of OCH_2Ph), 135.4 (C-6), 128.5 (Ar-C), 128.4 (Ar-C), 127.8 (Ar-C), 110.0 (C-5), 83.9 (C-1′), 82.2 (d, $^3J_{C,P}$ = 7.2 Hz, C-4′), 73.6 (C-3′), 68.7 (d, $^2J_{C,P}$ = 5.4 Hz, 2 OCH_2Ph), 67.1 (d, $^2J_{C,P}$ = 5.9 Hz, C-5′), 52.1 (αC-Lys), 51.8 (OCH_3), 50.4 (αC-Met), 47.5 (αC-Ala), 40.2 (εC-Lys), 36.0 (C-2′), 32.2 (βC-Met), 31.6 (βC-Lys), 29.3 (γC-Met), 29.1 (δC-Lys), 22.5 (γC-Lys), 16.8 (CH_3-Ala), 14.6 (-SCH_3), 12.0 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = –0.9 ppm. HRMS: calcd. for $C_{41}H_{55}N_6O_{14}PS$ [M – H]$^-$ 917.3161; found 917.3145.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Lys(Cbz)-OMe]-2′-deoxythymidine (28): Compound **28** was prepared according to the general procedure starting from **24** (326.3 mg, 0.489 mmol), tripeptide **5f** (408.1 mg, 0.587 mmol), and Et_3N (0.33 mL, 2.35 mmol) in dry CH_2Cl_2 (15 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:2, v/v; 92:4, v/v) to give conjugate **28** (347 mg, 64%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]-DMSO): δ = 11.53 (br. s, 1 H, N*H*-Thy), 8.31–8.25 (m, 2 H, 2 N*H*), 8.03–8.01 (m, 2 H, C*H*O and N*H*), 7.50 (s, 1 H, 6-H), 7.36–7.19 (m, 15 H, Ar-*H* of OBn), 7.23–7.19 (m, 1 H, N*H*), 6.18 (app t, *J* = 7.2 Hz, 1 H, 1′-H), 5.07–5.04 (m, 5 H, 3′-H and 2 POCH_2Ph-OBn), 4.99 (s, 2 H, OCH_2-Cbz), 4.47–4.39 (m, 1 H, α*H*-Met), 4.30–4.15 (m, 4 H, 2 α*H*-Lys, 5′-H, and 5′′-H), 4.09 (br. s, 1 H, 4′-H), 3.61 (s, 3 H, OCH_3), 2.99–2.92 (m, 4 H, 2 εCH_2-Lys), 2.41 (t, *J* = 7.8 Hz, 2 H, γCH_2-Met), 2.22–2.16 (m, 2 H, 2′-H and 2′′-H), 2.01 (s, 3 H, SCH_3), 1.90–1.76 (m, 2 H, βCH_2-Met), 1.69 (s, 3 H, CH_3-Thy), 1.67–1.49 (m, 4 H, 2 βCH_2-Lys), 1.42–1.35 (m, 4 H, 2 δCH_2-Lys), 1.33–1.17 (m, 4 H, 2 γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.4 (CO-Lys$_{C\text{-}Ter}$), 171.6 (CO-Met), 170.4 (CO-Lys$_{N\text{-}Ter}$), 163.5 (C-4), 161.0 (CHO), 155.2 (2 OCONH), 150.4 (C-2), 137.2 (C-6), 135.9 (d, $^3J_{C,P}$ = 6.7 Hz, 1C of OCH_2Ph), 135.4 (1C of Ph-Cbz), 128.5 (Ar-C), 128.4 (Ar-C), 128.3 (Ar-C), 127.8 (Ar-C), 127.7 (Ar-C), 127.6 (Ar-C), 110.0 (C-5), 83.9 (C-1′), 82.2 (d, $^3J_{C,P}$ = 8.2 Hz, C-4′), 73.6 (C-3′), 68.8–68.7 (3 OCH_2Ph), 65.1 (d, $^2J_{C,P}$ = 3.7 Hz, C-5′), 52.3 (αC-Lys), 52.6 (αC-Lys and OCH_3), 50.4 (αC-Met), 39.5 (2 εC-Lys merged with [D$_6$]DMSO), 36.0 (C-2′), 32.1 (βC-Met), 31.8 (2 βC-Lys), 30.5 (γC-Met), 29.3 (δC-Lys), 28.9 (δC-Lys), 22.5 (2 γC-Lys), 14.5 (SCH_3), 12.0 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, [D$_6$]DMSO): δ = –0.9 ppm. HRMS: calcd. for $C_{52}H_{68}N_7O_{16}PS$ [M – H]$^-$ 1108.4108; found 1108.4100.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-Boc-L-Ala-L-Lys(ε-carbamate)-L-Ala-OMe]-2′-deoxythymidine (29): Compound **29** was prepared according to the general procedure starting from **24** (505 mg, 0.756 mmol), tripeptide **13** (350 mg, 0.89 mmol), and Et_3N (0.16 mL, 1.13 mmol) in dry CH_2Cl_2 (20 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:2, v/v; 92:4, v/v) to give conjugate **29** (619 mg, 88%) as a colourless foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.41 (br. s, 1 H, N*H*-Thy), 7.44 (s, 1 H, 6-H), 7.33 (br. s, 10 H, Ar-*H* of OBn), 7.06 (br. s, 2 H, N*H*-Ala$_{C\text{-}Ter}$ and N*H*-Lys), 6.29 (dd, *J* = 9.1, 5.3 Hz, 1 H, 1′-H), 5.62 (br. s, 1 H, ε-N*H*-Lys), 5.39 (br. s, 1 H, N*H*-Ala$_{N\text{-}Ter}$), 5.10–5.01 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.55–4.48 (m, 2 H, α*H*-Ala$_{C\text{-}Ter}$ and α*H*-Lys), 4.29–4.26 (m, 2 H, 5′-H), 4.21–4.19 (m, 2 H, α*H*-Ala$_{N\text{-}Ter}$ and 5′′-H), 4.16 (br. s, 1 H, 4′-H), 3.74 (s, 3 H, OCH_3), 3.58–3.33 (m, 2 H, εCH_2-Lys), 2.31–2.25 (m, 2 H, 2′-H), 1.93–1.86 (m, 2 H, 2′′-H and βC$H_{2''}$-Lys), 1.84 (s, 3 H, CH_3-Thy), 1.71–1.63 (m, 1 H, βC$H_{2'''}$-Lys), 1.57–1.48 (m, 1 H, δCH_2-Lys), 1.42 (s, 9 H, *t*Bu), 1.40–1.34 (m, 8 H, γCH_2-Lys, 2 CH_3-Ala) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.4 (CO-Ala$_{C\text{-}Ter}$), 173.2 (CO-Ala$_{N\text{-}Ter}$), 171.3 (CO-Lys), 163.9 (C-4), 155.7 (2 OCONH), 150.8 (C-2), 135.5 (d, $^3J_{C,P}$ = 5.6 Hz, 1C of OCH_2Ph), 135.2 (C-6), 129.0 (Ar-C), 128.8 (Ar-C), 128.2 (Ar-C), 111.9 (C-5), 84.6 (C-1′), 83.1 (d, $^3J_{C,P}$ = 8.0 Hz, C-4′), 80.2 (1C *t*Bu), 75.0 (C-3′), 69.9–69.8 (2 d, $^2J_{C,P}$ = 5.4 Hz, 2 OCH_2Ph), 67.4 (d, $^2J_{C,P}$ = 5.8 Hz, C-5′), 52.8 (αC- Ala$_{C\text{-}Ter}$), 52.6 (OCH_3), 48.2 (αC-Lys and αC-Ala$_{N\text{-}Ter}$), 40.6 (εC-Lys), 37.4 (C-2′), 29.1 (βC-Lys), 28.4 (δC-Lys and CH_3-*t*Bu), 22.4 (γC-Lys), 18.0 (2 CH_3-Ala), 12.4 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, $CDCl_3$): δ = –0.7 ppm. HRMS: calcd. for $C_{43}H_{59}N_6O_{15}P$ [M – H]$^-$ 929.3703; found 929.3715.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-OMe]-2′-deoxythymidine (30): Compound **30** was prepared according to the general procedure starting from **24** (246.2 mg, 0.367 mmol), dipeptide **8** (168.3 mg, 0.3882 mmol), and Et_3N (0.22 mL, 1.55 mmol) in dry CH_2Cl_2 (15 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:4, v/v) to give conjugate **30** (276.5 mg, 84%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.30 (br. s, 1 H, N*H*-Thy), 8.20 (s, 1 H, C*H*O), 7.56 (d, *J* = 7.9 Hz, 1 H, N*H*-Met), 7.46 (s, 1 H, 6-H), 7.37–7.34 (m, 11 H, Ar-*H* of OBn and αN*H*-Lys), 6.30 (dd, *J* = 9.0, 5.3 Hz, 1 H, 1′-H), 5.95 (t, *J* = 5.5 Hz, εN*H*-Lys), 5.12–5.04 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.76–4.69 (m, 1 H, α*H*-Met), 4.61–4.54 (m, 1 H, α*H*-Lys), 4.27–4.22 (unresolved m, 3 H, 4′-H, 5′-H, and 5′′-H), 3.73 (s, 3 H, OCH_3), 3.18–3.11 (m, 2 H, εCH_2-Lys), 2.57 (t, *J* = 7.2 Hz, 2 H, γCH_2-Met), 2.30–2.24 (m, 1 H, 2′-H), 2.17–1.98 (m, 5 H, SCH_3 and βCH_2-Met), 1.92–1.65 (m, 6 H, CH_3-Thy, 2′′-H, and βCH_2-Lys), 1.58–1.48 (m, 2 H, δCH_2-Lys), 1.42–1.32 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.5 (CO-Lys), 171.2 (CO-Met), 164.1 (C-4), 161.7 (CHO), 155.6 (OCONH), 151.1 (C-2), 135.4 (d, $^3J_{C,P}$ = 6.2 Hz, 1C of OCH_2Ph), 135.1 (C-6), 128.8 (Ar-C), 128.7 (Ar-C), 128.0 (Ar-C), 111.9 (C-5), 84.4 (C-1′), 83.0 (d, $^3J_{C,P}$ = 8.0 Hz, C-4′), 74.7 (C-3′), 69.7 (2 d, $^2J_{C,P}$ = 5.5 Hz, 2 OCH_2Ph), 67.3 (d, $^2J_{C,P}$ = 5.4 Hz, C-5′), 52.4 (OCH_3), 52.0 (αC-Lys), 51.0 (αC-Met), 40.4 (εC-Lys), 37.3 (C-2′), 31.5 (βC-Met),

29.9 (βC-Lys and γC-Met), 28.8 (δC-Lys), 22.4 (γC-Lys), 15.2 (-SCH_3), 12.3 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.8 ppm. HRMS: calcd. for $C_{31}H_{44}N_5O_{13}PS$ [M – H]$^-$ 756.2320; found 756.2347.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Ala-L-Phe-OMe]-2′-deoxythymidine (31): Compound **31** was prepared according to the general procedure starting from **24** (246.2 mg, 0.367 mmol), tetrapeptide **9** (253 mg, 0.3882 mmol), and Et_3N (0.22 mL, 1.55 mmol) in dry CH_2Cl_2 (20 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:5, v/v) to give conjugate **31** (339 mg, 82%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]-DMSO): δ = 11.36 (br. s, 1 H, N*H*-Thy), 8.30–8.25 (m, 2 H, N*H*-Phe and N*H*-Met), 8.05 (d, J = 8.0 Hz, 1 H, αN*H*-Lys), 8.02 (s, 1 H, C*H*O), 7.91 (d, J = 7.5 Hz, 1 H, αN*H*-Ala), 7.50 (s, 1 H, 6-H), 7.36–7.32 (m, 11 H, Ar-*H* of OBn and εN*H*-Lys), 7.29–7.19 (m, 5 H, Ar-*H* of Phe), 6.19 (app t, J = 7.3 Hz, 1 H, 1′-H), 5.07–5.04 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.49–4.40 (m, 2 H, α*H*-Met and α*H*-Phe), 4.33–4.18 (unresolved m, 4 H, α*H*-Ala, α*H*-Lys, 5′-H, and 5′′-H), 4.12–4.08 (m, 1 H, 4′-H), 3.57 (s, 3 H, OCH_3), 3.05–2.90 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.43 (t, J = 7.8 Hz, 2 H, γCH_2-Met), 2.23–2.17 (m, 2 H, 2′-H and 2′′-H), 2.02 (s, 3 H, SCH_3), 1.95–1.75 (m, 2 H, βCH_2-Met), 1.70 (s, 3 H, CH_3-Thy), 1.64–1.45 (m, 2 H, βCH_2-Lys), 1.41–1.34 (m, 2 H, δCH_2-Lys), 1.29–1.22 (m, 2 H, γCH_2-Lys), 1.17 (d, J = 6.9 Hz, 3 H, CH_3-Ala) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.2 (CO-Ala), 171.7 (CO-Phe), 171.0 (CO-Lys), 170.6 (CO-Met), 163.6 (C-4), 161.0 (CHO), 155.2 (OCONH), 150.4 (C-2), 136.9 (1C of Phe), 135.9 (d, $^3J_{C,P}$ = 6.9 Hz, 1C of OCH_2Ph), 135.4 (C-6), 129.0 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 128.2 (Ar-C), 127.8 (Ar-C), 126.5 (Ar-C), 110.0 (C-5), 83.9 (C-1′), 82.2 (d, $^3J_{C,P}$ = 7.4 Hz, C-4′), 73.6 (C-3′), 68.7 (2 d, $^2J_{C,P}$ = 5.4 Hz, 2 OCH_2Ph), 67.0 (d, $^2J_{C,P}$ = 4.9 Hz, C-5′), 53.5 (αC-Phe), 52.4 (αC-Lys), 51.8 (OCH_3), 50.4 (αC-Met), 47.8 (αC-Ala), 40.2 (εC-Lys), 36.6 (βC-Phe), 36.0 (C-2′), 32.2 (βC-Met), 31.5 (βC-Lys), 29.3 (γC-Met), 29.0 (δC-Lys), 22.6 (γC-Lys), 18.2 (CH_3-Ala), 14.6 (-SCH_3), 12.0 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, [D$_6$]DMSO): δ = –0.9 ppm. HRMS: calcd. for $C_{50}H_{64}N_7O_{15}PS$ [M – H]$^-$ 1064.3846; found 1064.3867.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Ala-L-Ala-L-Phe-OMe]-2′-deoxythymidine (32): Compound **32** was prepared according to the general procedure starting from **24** (246.2 mg, 0.367 mmol), pentapeptide **10** (281 mg, 0.3882 mmol), and Et_3N (0.22 mL, 1.55 mmol) in dry CH_2Cl_2 (20 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:6, v/v) to give conjugate **32** (380 mg, 86%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.36 (br. s, 1 H, N*H*-Thy), 8.29 (d, J = 8.6 Hz, 1 H, N*H*-Met), 8.23 (d, J = 7.5 Hz, 1 H, N*H*-Phe), 8.07 (d, J = 7.6 Hz, 1 H, αN*H*-Lys), 8.02 (s, 1 H, C*H*O), 7.96 (d, J = 7.3 Hz, 1 H, αN*H*-Ala), 7.86 (d, J = 7.6 Hz, 1 H, αN*H*-Ala), 7.50 (s, 1 H, 6-H), 7.36–7.32 (m, 11 H, Ar-*H* of OBn and εN*H*-Lys), 7.29–7.19 (m, 5 H, Ar-*H* of Phe), 6.19 (app t, J = 7.3 Hz, 1 H, 1′-H), 5.07–5.04 (m, 5 H, 3′-H and 2 OCH_2Ph), 4.49–4.40 (m, 2 H, α*H*-Met and α*H*-Phe), 4.29–4.19 (unresolved m, 5 H, 2 α*H*-Ala, α*H*-Lys, 5′-H, and 5′′-H), 4.12–4.08 (m, 1 H, 4′-H), 3.57 (s, 3 H, OCH_3), 3.05–2.89 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.43 (t, J = 7.9 Hz, 2 H, γCH_2-Met), 2.25–2.17 (m, 2 H, 2′-H and 2′′-H), 2.03 (s, 3 H, SCH_3), 1.96–1.75 (m, 2 H, βCH_2-Met), 1.69 (s, 3 H, CH_3-Thy), 1.67–1.46 (m, 2 H, βCH_2-Lys), 1.43–1.34 (m, 2 H, δCH_2-Lys), 1.31–1.22 (m, 2 H, γCH_2-Lys), 1.20–1.13 (m, 6 H, 2 CH_3-Ala) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.1 (CO-Ala$_{C\text{-}ter}$), 171.7 (CO-Phe), 171.5 (CO-Ala$_{N\text{-}ter}$), 171.1 (CO-Lys), 170.6 (CO-Met), 163.5 (C-4), 161.0 (CHO), 155.4 (OCONH), 150.4 (C-2), 137.0 (1C of Phe), 135.9 (d, $^3J_{C,P}$ = 6.9 Hz, 1C of OCH_2Ph), 135.4 (C-6), 129.0 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 128.2 (Ar-C), 127.8 (Ar-C), 126.5 (Ar-C), 110.0 (C-5), 83.9 (C-1′), 82.2 (d, $^3J_{C,P}$ = 7.3 Hz, C-4′), 73.6 (C-3′), 68.7 (2 d, $^2J_{C,P}$ = 5.4 Hz, 2 OCH_2Ph), 67.0 (d, $^2J_{C,P}$ = 5.3 Hz, C-5′), 53.5 (αC-Phe), 52.5 (αC-Lys), 51.8 (OCH_3), 50.4 (αC-Met), 47.9 (αC-Ala), 47.7 (αC-Ala), 40.2 (εC-Lys), 36.6 (βC-Phe), 36.0 (C-2′), 32.2 (βC-Met), 31.4 (βC-Lys), 29.3 (γC-Met), 29.0 (δC-Lys), 22.7 (γC-Lys), 18.2 (CH_3-Ala), 17.9 (CH_3-Ala), 14.6 (-SCH_3), 12.0 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, [D$_6$]DMSO): δ = –0.9 ppm. HRMS: calcd. for $C_{53}H_{69}N_8O_{16}PS$ [M – H]$^-$ 1135.4217; found 1135.4203.

General Procedure for the Synthesis of 3′-*O*-(Peptide carbamate)-2′-deoxythymidine-5′-monophosphate Salts (19 and 33–40): Pd/C (10%; Degussa; 10–100% w/w) was added to a stirred solution of 5′-*O*-(dibenzylphosphate)-3′-*O*-(peptide carbamate)-2′deoxythymidine (1 equiv.) in EtOH or MeOH, and the mixture was hydrogenated at atmospheric pressure using a balloon filled with H_2 for 24 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The resulting crude residue was purified RP-HPLC [TEAB (triethylammonium bicarbonate; 50 mmol) in H_2O/MeCN, 98:2; and TEAB (50 mmol) in H_2O/MeCN, 50:50]. The collected eluate was freeze-dried repeatedly until constant mass to give the desired 3′-*O*-(peptide carbamate)-2′-deoxythymidine-5′-monophosphate triethylammonium salts.

3′-*O*-[*N*-For-Gly-L-β-amino-Ala-(β-carbamate)-L-Phe-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (33): Compound **33** was obtained as a white solid (149 mg, 81%) according to the general procedure, starting from a stirred solution of **25** (180 mg, 0.205 mmol), Et_3N (0.057 mL, 0.410 mmol), and Pd/C (10%; Degussa; 18 mg, 10% w/w) in MeOH (15 mL). ^{1}H NMR (500 MHz, D_2O): δ = 8.16 (s, 1 H, C*H*O), 7.79 (s, 1 H, 6-H), 7.31–7.19 (m, 5 H, Ar-*H* of Phe), 6.32 (dd, J = 8.4, 6.6 Hz, 1 H, 1′-H), 5.22–5.21 (m, 1 H, 3′-H), 4.72–4.69 (m, 1 H, α*H*-Phe), 4.48–4.45 (m, 1 H, α*H*-β-amino-Ala), 4.30–4.29 (m, 1 H, 4′-H), 4.05–4.03 (m, 2 H, 5′-H and 5′′-H), 3.91 (d, J = 3.4 Hz, 2 H, CH_2-Gly), 3.70 (s, 3 H, OCH_3), 3.44–3.24 (m, 2 H, βCH_2-β-amino-Ala), 3.22–2.95 (m, 2 H, βCH_2-Phe), 2.39–2.36 (m, 2 H, 2′-H and 2′′-H), 1.88 (s, 3 H, CH_3-Thy) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 172.4 (CO-Phe), 170.3 (CO-β-amino-Ala), 170.1 (CO-Gly), 165.9 (C-4), 164.2 (CHO), 156.9 (OCONH), 151.1 (C-2), 136.6 (C-6), 135.6 (1C of Ph), 128.6 (Ar-C), 128.0 (Ar-C), 126.5 (Ar-C), 111.3 (C-5), 84.1 (C-1′), 83.0 (d, $^3J_{C,P}$ = 8.7 Hz, C-4′), 75.8 (C-3′), 64.3 (d, $^2J_{C,P}$ = 5.5 Hz, C-5′), 53.4 (αC-Phe), 52.7 (αC-β-amino-Ala), 52.3 (OCH_3), 40.6 (βC-β-amino-Ala), 40.3 (αC-Gly), 36.0 (βC-Phe), 35.9 (C-2′), 11.0 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 0.3 ppm. HRMS: calcd. for $C_{27}H_{35}N_6O_{14}P$ [M – H]$^-$ 697.1876; found 697.1884.

3′-*O*-[*N*-For-Gly-L-Lys-(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (19): Compound **19** was obtained as a white solid (255 mg, 83%) according to the general procedure, starting from a stirred solution of **18** (300 mg, 0.326 mmol), Et_3N (0.091 mL, 0.651 mmol), and Pd/C (10%; Degussa; 30 mg, 10% w/w) in MeOH (25 mL). ^{1}H NMR (500 MHz, D_2O): δ = 8.17 (s, 1 H, C*H*O), 7.85 (s, 1 H, 6-H), 7.35–7.19 (m, 5 H, Ar-H of Phe), 6.32 (dd, J = 7.5, 6.1 Hz, 1 H, 1′-H), 5.26–5.23 (m, 1 H, 3′-H), 4.73–4.70 (m, 1 H, α*H*-Phe), 4.31 (br. s, 1 H, 4′-H), 4.27–4.21 (m, 1 H, α*H*-Lys), 4.04–4.00 (m, 2 H, 5′-H and 5′′-H), 3.96 (s, 2 H, CH_2-Gly), 3.73 (s, 3 H, OCH_3), 3.12–3.10 (m, 2 H, εCH_2-Lys), 3.08–3.02 (m, 2 H, βCH_2-Phe), 2.45–2.40 (m, 2 H, 2′-H and 2′′-H), 1.93 (s, 3 H, CH_3-Thy), 1.69–1.58 (m, 2 H, βCH_2-Lys), 1.51–1.44 (m, 2 H, δCH_2-Lys), 1.29–1.26 (m, 2 H, γCH_2-Lys merged with Et_3N) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 177.2

(CO-Phe), 175.3 (CO-Lys), 172.7 (CO-Gly), 172.5 (C-4), 170.5 (CHO), 164.4 (OCONH), 157.3 (C-2), 137.2 (C-6), 136.6 (1C of Ph), 129.1 (Ar-C), 128.2 (Ar-C), 126.5 (Ar-C), 111.8 (C-5), 84.6 (C-1′), 83.0 (d, $^3J_{C,P}$ = 7.5 Hz, C-4′), 75.8 (C-3′), 63.7 (d, $^2J_{C,P}$ = 4.0 Hz, C-5′), 55.7 (αC-Phe), 53.6 (αC-Lys), 53.5 (OCH_3), 43.3 (αC-Gly), 40.7 (εC-Lys), 37.5 (C-2′), 36.2 (βC-Phe), 30.3 (βC-Lys), 28.0 (δC-Lys), 22.0 (γC-Lys), 12.1 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 3.8 ppm. HRMS: calcd. for $C_{30}H_{41}N_6O_{14}P$ [M – H]$^-$ 739.2345; found 739.2336.

3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Phe-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (34): Compound **34** was obtained as a white solid (239 mg, 78%) according to the general procedure, starting from a stirred solution of **26** (300 mg, 0.301 mmol), Et_3N (0.084 mL, 0.603 mmol), and Pd/C (10%; Degussa; 240 mg, 80% w/w) in MeOH (25 mL). ^{1}H NMR (600 MHz, D_2O): δ = 11.29 (br. s, 1 H, N*H*-Thy), 8.43 (d, *J* = 7.1 Hz, 1 H, N*H*-Lys), 8.35 (d, *J* = 8.3 Hz, 1 H, N*H*-Met), 8.01 (s, 1 H, C*H*O), 7.90 (s, 1 H, 6-H), 7.34 (t, *J* = 5.5 Hz, εN*H*-Lys), 6.23 (dd, *J* = 9.0, 5.6 Hz, 1 H, 1′-H), 5.14–5.13 (m, 1 H, 3′-H), 4.49–4.45 (m, 1 H, α*H*-Met), 4.21–4.17 (m, 1 H, α*H*-Lys), 4.05 (br. s, 1 H, 4′-H), 3.86 (br. s, 2 H, 5′-H and 5′′-H), 3.62 (s, 3 H, OCH_3), 2.99–2.96 (m, 2 H, εCH_2-Lys), 2.44 (t, *J* = 7.9 Hz, 2 H, γCH_2-Met), 2.30–2.13 (m, 2 H, 2′-H and 2′′-H), 2.04 (s, 3 H, SCH_3), 1.93–1.77 (m, 5 H, βCH_2-Met and CH_3-Thy), 1.72–1.60 (m, 2 H, βCH_2-Lys), 1.41–1.37 (m, 2 H, δCH_2-Lys), 1.32–1.27 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 172.8 (CO-Lys), 172.5 (CO-Phe), 172.1 (CO-Met), 165.9 (C-4), 163.4 (CHO), 156.9 (OCONH), 151.1 (C-2), 136.5 (C-6), 135.6 (1C of Phe), 128.5 (Ar-C), 128.0 (Ar-C), 126.5 (Ar-C), 111.3 (C-5), 84.2 (C-1′), 83.1 (d, $^3J_{C,P}$ = 9.0 Hz, C-4′), 75.2 (C-3′), 64.4 (d, $^2J_{C,P}$ = 4.0 Hz, C-5′), 53.4 (αC-Phe), 52.9 (αC-Lys), 52.2 (OCH_3), 50.6 (αC-Met), 39.3 (εC-Lys), 36.1 (C-2′), 35.9 (βC-Phe), 30.0 (βC-Lys), 29.7 (βC-Met), 28.3 (γC-Met), 27.5 (δC-Lys), 21.4 (γC-Lys), 13.4 (-SCH_3), 11.0 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = –0.2 ppm. HRMS: calcd. for $C_{33}H_{47}N_6O_{14}PS$ [M – H]$^-$ 813.2536; found 813.2543.

3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Ala-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (35): Compound **35** was obtained as a white solid (148 mg, 76%) according to the general procedure, starting from a stirred solution of **27** (190 mg, 0.206 mmol), Et_3N (0.058 mL, 0.413 mmol), and Pd/C (10%; Degussa; 95 mg, 50% w/w) in MeOH (25 mL). ^{1}H NMR (500 MHz, D_2O): δ = 8.21 (s, 1 H, C*H*O), 7.97 (s, 1 H, 6-H), 6.44 (dd, *J* = 8.6, 6.3 Hz, 1 H, 1′-H), 5.35–5.34 (m, 1 H, 3′-H), 4.63–4.61 (m, 1 H, α*H*-Met), 4.50–4.45 (q, *J* = 7.2 Hz, 1 H, α*H*-Ala), 4.41–4.38 (m, 2 H, 4′-H and α*H*-Lys), 4.11 (br. s, 2 H, 5′-H and 5′′-H), 3.84 (s, 3 H, OCH_3), 3.24 (t, *J* = 6.4 Hz, 2 H, εCH_2-Lys), 2.69–2.64 (m, 2 H, γCH_2-Met), 2.58–2.50 (m, 2 H, 2′-H and 2′′-H), 2.19 (s, 3 H, SCH_3), 2.18–2.07 (m, 2 H, βCH_2-Met), 2.02 (s, 3 H, CH_3-Thy), 1.94–1.80 (m, 2 H, βCH_2-Lys), 1.65–1.59 (m, 2 H, δCH_2-Lys), 1.53–1.48 (m, 5 H, γCH_2-Lys and CH_3-Ala) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 174.3 (CO-Ala), 173.0 (CO-Lys), 172.5 (CO-Met), 166.1 (C-4), 163.5 (CHO), 157.0 (OCONH), 151.3 (C-2), 136.9 (C-6), 111.4 (C-5), 84.2 (C-1′), 83.5 (d, $^3J_{C,P}$ = 9.2 Hz, C-4′), 75.5 (C-3′), 63.8 (d, $^2J_{C,P}$ = 3.4 Hz, C-5′), 53.0 (αC-Lys), 52.2 (OCH_3), 50.7 (αC-Met), 48.1 (αC-Ala), 39.4 (εC-Lys), 36.0 (C-2′), 29.9 (βC-Met and βC-Lys), 28.3 (γC-Met), 27.7 (δC-Lys), 21.4 (γC-Lys), 15.2 (CH_3-Ala), 13.5 (SCH_3-Met), 11.1 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 2.0 ppm. HRMS: calcd. for $C_{27}H_{43}N_6O_{14}PS$ [M – H]$^-$ 737.2222; found 737.2228.

3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Lys(ε-NH_2)-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Hydrochloride Salt (36): Compound **36** was prepared according to the general procedure starting from a stirred solution of **28** (300 mg, 0.270 mmol) and $Pd(OH)_2$/C (20%; 150 mg, 50% w/w) in EtOH/H_2O, 10:1 (25 mL). After HPLC purification, the resulting triethylammonium salt was further acidified with HCl to stabilize the free amino group to give **36** (173.5 mg, 58%) as a white solid. ^{1}H NMR (600 MHz, D_2O): δ = 8.11 (s, 1 H, C*H*O), 7.78 (s, 1 H, 6-H), 6.34 (dd, *J* = 8.6, 6.2 Hz, 1 H, 1′-H), 5.23–5.22 (m, 1 H, 3′-H), 4.49–4.47 (m, 1 H, α*H*-Met), 4.38–4.28 (m, 4 H, 2 α*H*-Lys and 4′-H), 4.09 (br. s, 1 H, 5′-H and 5′′-H), 3.73 (s, 3 H, OCH_3), 3.13–3.10 (m, 2 H, εCH_2-Lys), 2.98–2.94 (m, 2 H, εCH_2-Lys), 2.57–2.50 (m, 2 H, γCH_2-Met), 2.43–2.36 (m, 2 H, 2′-H and 2′′-H), 2.07 (s, 3 H, SCH_3), 2.05–1.98 (m, 2 H, βCH_2-Met), 1.89 (s, 3 H, CH_3-Thy), 1.88–1.61 (m, 8 H, 2 βCH_2-Lys and 2 δCH_2-Lys), 1.52–1.36 (m, 4 H, 2 γCH_2-Lys) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 175.0 ($CO\text{-}Lys_{N\text{-}Ter}$), 173.7 ($CO\text{-}Lys_{C\text{-}Ter}$), 172.9 (CO-Met), 166.4 (C-4), 164.0 (CHO), 157.3 (OCONH), 151.5 (C-2), 136.9 (C-6), 111.7 (C-5), 84.7 (C-1′), 83.5 (d, $^3J_{C,P}$ = 8.6 Hz, C-4′), 75.5 (C-3′), 64.9 (d, $^2J_{C,P}$ = 4.0 Hz, C-5′), 53.5 (αC-$Lys_{N\text{-}Ter}$), 52.6 (OCH_3), 52.2 (αC-$Lys_{C\text{-}Ter}$), 51.3 (αC-Met), 39.7 (εC-Lys), 36.4 (C-2′), 30.4 (βC-$Lys_{N\text{-}Ter}$), 30.2 (βC-Met), 29.6 (βC-$Lys_{N\text{-}Ter}$), 28.7 (γC-Met), 25.9 (2 δC-Lys), 21.9 (2 γC-Lys), 13.7 (SCH_3), 11.4 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = –0.2 ppm. HRMS: calcd. for $C_{52}H_{68}N_7O_{16}PS$ [M – H]$^-$ 794.2801; found 794.2811.

3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (38): Compound **38** was obtained as a white solid (217 mg, 79%) according to the general procedure, starting from a stirred solution of **30** (240 mg, 0.316 mmol), Et_3N (0.088 mL, 0.632 mmol), and Pd/C (10%; Degussa; 120 mg, 50% w/w) in MeOH (25 mL). ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 11.29 (br. s, 1 H, N*H*-Thy), 8.43 (d, *J* = 7.1 Hz, 1 H, N*H*-Lys), 8.35 (d, *J* = 8.3 Hz, 1 H, N*H*-Met), 8.01 (s, 1 H, C*H*O), 7.90 (s, 1 H, 6-H), 7.34 (t, *J* = 5.5 Hz, εN*H*-Lys), 6.23 (dd, *J* = 9.0, 5.6 Hz, 1 H, 1′-H), 5.14–5.13 (m, 1 H, 3′-H), 4.49–4.45 (m, 1 H, α*H*-Met), 4.21–4.17 (m, 1 H, α*H*-Lys), 4.05 (br. s, 1 H, 4′-H), 3.86 (br. s, 2 H, 5′-H and 5′′-H), 3.62 (s, 3 H, OCH_3), 2.99–2.96 (m, 2 H, εCH_2-Lys), 2.44 (t, *J* = 7.9 Hz, 2 H, γCH_2-Met), 2.30–2.13 (m, 2 H, 2′-H and 2′′-H), 2.04 (s, 3 H, SCH_3), 1.93–1.77 (m, 5 H, βCH_2-Met and CH_3-Thy), 1.72–1.60 (m, 2 H, βCH_2-Lys), 1.41–1.37 (m, 2 H, δCH_2-Lys), 1.32–1.27 (m, 2 H, γCH_2-Lys) ppm. ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 172.5 (CO-Lys), 171.1 (CO-Met), 163.8 (C-4), 161.0 (CHO), 155.4 (OCONH), 150.7 (C-2), 136.2 (C-6), 110.2 (C-5), 83.8 (C-1′ and C-4′), 75.3 (C-3′), 64.3 (d, $^2J_{C,P}$ = 5.2 Hz, C-5′), 52.1 (αC-Lys), 51.9 (OCH_3), 50.2 (αC-Met), 39.6 (εC-Lys), 36.7 (C-2′), 32.3 (βC-Met), 30.3 (βC-Lys), 29.2 (γC-Met), 28.9 (δC-Lys), 22.8 (γC-Lys), 14.7 (-SCH_3), 12.1 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, [D_6]DMSO): δ = –0.3 ppm. HRMS: calcd. for $C_{24}H_{38}N_5O_{13}PS$ [M – H]$^-$ 666.1851; found 666.1854.

3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Ala-L-Phe-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (39): Compound **39** was obtained as a white solid (232 mg, 81%) according to the general procedure, starting from a stirred solution of **31** (280 mg, 0.263 mmol), Et_3N (0.073 mL, 0.525 mmol), and Pd/C (10%; Degussa; 224 mg, 80% w/w) in MeOH (30 mL). ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 11.28 (br. s, 1 H, N*H*-Thy), 8.37 (d, *J* = 7.8 Hz, 1 H, N*H*-Met), 8.27 (d, *J* = 7.5 Hz, 1 H, N*H*-Phe), 8.09 (d, *J* = 7.8 Hz, 1 H, αN*H*-Lys), 8.02 (s, 1 H, C*H*O), 7.95 (d, *J* = 7.7 Hz, 1 H, αN*H*-Ala), 7.89 (s, 2 H, 6-H), 7.31 (d, *J* = 5.4 Hz, 1 H, εN*H*-Lys), 7.28–7.19 (m, 5 H, Ar-*H* of Phe), 6.23 (dd, *J* = 8.7, 5.9 Hz, 1 H, 1′-H), 5.14–5.13 (m, 1 H, 3′-H), 4.47–4.40 (m, 2 H, α*H*-Met and α*H*-Phe), 4.29–4.24 (m, 1 H, α*H*-Ala), 4.21–4.17 (m, 1 H, α*H*-Lys), 4.05 (br. s, 1 H, 4′-H), 3.89–3.86 (m, 2 H, 5′-H and 5′′-H), 3.57 (s, 3 H, OCH_3), 3.03–2.89 (m, 4 H, εCH_2-Lys and

βCH_2-Phe), 2.43 (t, J = 8.0 Hz, 2 H, γCH_2-Met), 2.29–2.11 (m, 2 H, 2′-H and 2′′-H), 2.02 (s, 3 H, SCH_3), 1.93–1.73 (m, 5 H, βCH_2-Met and CH_3-Thy), 1.64–1.45 (m, 2 H, βCH_2-Lys), 1.40–1.34 (m, 2 H, δCH_2-Lys), 1.30–1.20 (m, 2 H, γCH_2-Lys), 1.20–1.16 (m, 3 H, CH_3-Ala) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 172.3 (CO-Ala), 171.8 (CO-Phe), 171.1 (CO-Lys), 170.7 (CO-Met), 163.8 (C-4), 161.1 (CHO), 155.4 (OCONH), 150.6 (C-2), 137.1 (1C of Phe), 136.2 (C-6), 129.1 (Ar-C), 128.3 (Ar-C), 126.6 (Ar-C), 110.2 (C-5), 83.7 (C-1′ and C-4′), 75.2 (C-3′), 64.3 (d, $^2J_{C,P}$ = 5.0 Hz, C-5′), 53.6 (αC-Phe), 52.5 (αC-Lys), 51.9 (OCH_3), 50.5 (αC-Met), 47.9 (αC-Ala), 40.2 (εC-Lys), 36.7 (C-2′), 36.6 (βC-Phe), 32.1 (βC-Met), 31.5 (βC-Lys), 29.4 (γC-Met), 29.1 (δC-Lys), 22.7 (γC-Lys), 18.2 (CH$_3$-Ala), 14.6 (-SCH$_3$), 12.1 (CH$_3$-Thy) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = –0.3 ppm. HRMS: calcd. for $C_{36}H_{52}N_7O_{15}PS$ [M – H]$^-$ 884.2907; found 884.2920.

3′-*O*-[*N*-For-L-Met-L-Lys(ε-carbamate)-L-Ala-L-Ala-L-Phe-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (40): Compound **40** was obtained as a white solid (254 mg, 78%) according to the general procedure, starting from a stirred solution of **32** (320 mg, 0.281 mmol), Et$_3$N (0.078 mL, 0.563 mmol), and Pd/C (10%; Degussa; 256 mg, 80% w/w) in MeOH (30 mL). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 11.28 (br. s, 1 H, NH-Thy), 8.37 (d, J = 8.2 Hz, 1 H, NH-Met), 8.28 (d, J = 7.5 Hz, 1 H, NH-Phe), 8.10 (d, J = 7.7 Hz, 1 H, αNH-Lys), 8.02 (s, 1 H, CHO), 7.99 (d, J = 7.4 Hz, 1 H, αNH-Ala), 7.89–7.86 (m, 2 H, 6-H and αNH-Ala), 7.31 (d, J = 5.5 Hz, 1 H, εNH-Lys), 7.28–7.19 (m, 5 H, Ar-H of Phe), 6.23 (dd, J = 8.8, 5.8 Hz, 1 H, 1′-H), 5.15–5.14 (m, 1 H, 3′-H), 4.47–4.41 (m, 2 H, αH-Met and αH-Phe), 4.30–4.25 (m, 1 H, αH-Ala$_{C\text{-}ter}$), 4.25–4.23 (m, 1 H, αH-Ala$_{N\text{-}ter}$), 4.22–4.17 (m, 1 H, αH-Lys), 4.05 (br. s, 1 H, 4′-H), 3.89–3.86 (m, 2 H, 5′-H and 5′′-H), 3.57 (s, 3 H, OCH_3), 3.03–2.92 (m, 4 H, εCH_2-Lys and βCH_2-Phe), 2.43 (t, J = 8.2 Hz, 2 H, γCH_2-Met), 2.30–2.12 (m, 2 H, 2′-H and 2′′-H), 2.03 (s, 3 H, SCH_3), 1.94–1.74 (m, 5 H, βCH_2-Met and CH_3-Thy), 1.68–1.47 (m, 2 H, βCH_2-Lys), 1.40–1.35 (m, 2 H, δCH_2-Lys), 1.30–1.21 (m, 2 H, γCH_2-Lys), 1.20–1.16 (m, 6 H, 2 CH_3-Ala) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 172.2 (CO-Ala$_{C\text{-}ter}$), 171.7 (CO-Phe), 171.5 (CO-Ala$_{N\text{-}ter}$), 171.2 (CO-Lys), 170.7 (CO-Met), 163.8 (C-4), 161.0 (CHO), 155.3 (OCONH), 150.4 (C-2), 137.0 (1C of Phe), 136.1 (C-6), 129.1 (Ar-C), 128.2 (Ar-C), 126.5 (Ar-C), 110.1 (C-5), 83.7 (C-1′ and C-4′), 75.2 (C-3′), 64.2 (d, $^2J_{C,P}$ = 4.6 Hz, C-5′), 53.6 (αC-Phe), 52.6 (αC-Lys), 51.8 (OCH_3), 50.4 (αC-Met), 48.0 (αC-Ala$_{C\text{-}ter}$), 47.8 (αC-Ala$_{N\text{-}ter}$), 40.2 (εC-Lys), 36.7 (C-2′), 36.6 (βC-Phe), 32.1 (βC-Met), 31.4 (βC-Lys), 29.3 (γC-Met), 29.1 (δC-Lys), 22.7 (γC-Lys), 18.2 (CH$_3$-Ala), 17.9 (CH$_3$-Ala), 14.6 (-SCH$_3$), 12.0 (CH$_3$-Thy) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = –0.3 ppm. HRMS: calcd. for $C_{39}H_{57}N_8O_{16}PS$ [M – H]$^-$ 955.3278; found 955.3293.

3′-*O*-[H-L-Ala-L-Lys(ε-carbamate)-L-Ala-OMe]-2′-deoxythymidine-5′-monophosphate TFA Salt (41): According to the general procedure, a stirred solution of **29** (400 mg, 0.476 mmol) and Pd/C (10%; Degussa; 40 mg, 10% w/w) in MeOH (15 mL) was hydrogenated to give crude 3′-*O*-[*N*-Boc-L-Ala-L-Lys-(ε-carbamate)-L-Ala-OMe]-2′-deoxythymidine-5′-monophosphate **37** (357 mg, quantitative) as a white solid, which was used in the next step without further purification.

Thioanisole (0.056 mL, 0.476 mmol) and then trifluoroacetic acid (1 mL) were added to a stirred solution of **37** (357 mg, 0.476 mmol) in H$_2$O (3 mL) at 0 °C. The reaction mixture was stirred at room temp. for 1.5 h, and then the volatiles were removed in vacuo. The crude residue was coevaporated with toluene (3×), and then purified by RP-Prep HPLC [TFA (0.05%) in H$_2$O/MeCN, 98:2; and TFA (0.05%) in MeCN/H$_2$O, 98:2]. The collected eluate was freeze-dried repeatedly until constant mass to give **41** (258 mg, 71% over two steps) as a white solid. ^{1}H NMR (500 MHz, D$_2$O): δ = 7.75 (s, 1 H, 6-H), 6.31 (dd, J = 8.7, 6.0 Hz, 1 H, 1′-H), 5.21–5.20 (m, 1 H, 3′-H), 4.35–4.30 (m, 1 H, αH-Ala$_{C\text{-}Ter}$), 4.29 (br. s, 1 H, 4′-H), 4.25–4.22 (m, 1 H, αH-Lys), 4.09–4.05 (m, 2 H, 5′-H and 5′′-H), 4.05–4.02 (m, 1 H, αH-Ala$_{N\text{-}Ter}$), 3.70 (s, 3 H, OCH_3), 3.12–3.10 (m, 2 H, εCH_2-Lys), 2.40–2.34 (m, 2 H, 2′-H and 2′′-H), 1.87 (s, 3 H, CH_3-Thy), 1.76–1.70 (m, 1 H, βCH_2-Lys), 1.52–1.44 (m, 5 H, δCH_2-Lys and CH_3-Ala$_{N\text{-}Ter}$), 1.43–1.33 (m, 5 H, γCH_2-Lys and CH_3-Ala$_{C\text{-}Ter}$) ppm. ^{13}C NMR (125 MHz, D$_2$O): δ = 174.2 (CO-Ala$_{C\text{-}Ter}$), 173.1 (CO-Lys), 170.1 (CO-Ala$_{N\text{-}Ter}$), 165.9 (C-4), 157.0 (OCONH), 151.2 (C-2), 136.5 (C-6), 111.3 (C-5), 84.3 (C-1′), 83.1 (d, $^3J_{C,P}$ = 9.0 Hz, C-4′), 75.2 (C-3′), 64.5 (d, $^2J_{C,P}$ = 2.8 Hz, C-5′), 53.1 (αC-Lys), 52.2 (OCH$_3$), 48.2 (αC-Ala$_{C\text{-}Ter}$), 48.1 (αC-Ala$_{N\text{-}Ter}$), 39.3 (εC-Lys), 36.0 (C-2′), 29.9 (βC-Lys), 27.8 (δC-Lys), 21.3 (γC-Lys), 15.9 (CH$_3$-Ala$_{N\text{-}Ter}$), 15.1 (CH$_3$-Ala$_{C\text{-}Ter}$), 11.0 (CH$_3$-Thy) ppm. ^{31}P NMR (202 MHz, D$_2$O): δ = –0.3 ppm. HRMS: calcd. for $C_{24}H_{39}N_6O_{13}P$ [M – H]$^-$ 649.2240; found 649.2241.

3′-*O*-[H-L-Ala-L-Lys(ε-carbamate)-L-Ala-OH]-2′-deoxythymidine-5′-monophosphate TFA Salt (42): LiOH (16.2 mg, 0.687 mmol) was added to a stirred solution of **41** (150 mg, 0.196 mmol) in THF/H$_2$O/MeOH, 1:1:1 (3 mL), and the resulting solution was stirred at room temp. for 3 h. The reaction mixture was neutralized with trifluoroacetic acid, and the volatiles were removed under reduced pressure. The crude residue was purified by RP-Prep HPLC [TFA (0.05%) in H$_2$O/MeCN, 98:2; and TFA (0.05%) in MeCN/H$_2$O, 98:2] to give **42** (109 mg, 74%) as a white solid. ^{1}H NMR (600 MHz, D$_2$O): δ = 7.77 (s, 1 H, 6-H), 6.33 (dd, J = 8.9, 5.9 Hz, 1 H, 1′-H), 5.22–5.21 (m, 1 H, 3′-H), 4.32–4.29 (m, 2 H, αH-Ala$_{N\text{-}Ter}$ and 4′-H), 4.27–4.25 (m, 1 H, αH-Lys), 4.09–4.08 (m, 2 H, 5′-H and 5′′-H), 4.07–4.05 (m, 1 H, αH-Ala$_{C\text{-}Ter}$), 3.12–3.10 (m, 2 H, εCH_2-Lys), 2.42–2.36 (m, 2 H, 2′-H and 2′′-H), 1.88 (s, 3 H, CH_3-Thy), 1.81–1.69 (m, 1 H, βCH_2-Lys), 1.53–1.46 (m, 5 H, δCH_2-Lys and CH_3-Ala$_{C\text{-}Ter}$), 1.44–1.36 (m, 5 H, γCH_2-Lys and CH_3-Ala$_{N\text{-}Ter}$) ppm. ^{13}C NMR (150 MHz, D$_2$O): δ = 176.0 (CO-Ala$_{C\text{-}Ter}$), 173.3 (CO-Lys), 170.4 (CO-Ala$_{N\text{-}Ter}$), 166.3 (C-4), 157.4 (OCONH), 151.5 (C-2), 136.9 (C-6), 111.7 (C-5), 84.7 (C-1′), 83.5 (d, $^3J_{C,P}$ = 9.0 Hz, C-4′), 75.5 (C-3′), 64.9 (d, $^2J_{C,P}$ = 3.4 Hz, C-5′), 53.4 (αC-Lys), 48.5 (αC-Ala$_{C\text{-}Ter}$ and αC-Ala$_{N\text{-}Ter}$), 39.7 (εC-Lys), 36.4 (C-2′), 30.3 (βC-Lys), 28.1 (δC-Lys), 21.7 (γC-Lys), 16.3 (CH$_3$-Ala$_{C\text{-}Ter}$), 15.7 (CH_3-Ala$_{N\text{-}Ter}$), 11.4 (CH$_3$-Thy) ppm. ^{31}P NMR (202 MHz, D$_2$O): δ = –0.3 ppm. HRMS: calcd. for $C_{23}H_{37}N_6O_{13}P$ [M – H]$^-$ 635.2083; found 635.2093.

5′-*O*-(4-Monomethoxytrityl)-*xylo*-2′-deoxythymidine (43): Et$_3$N (1.72 mL, 12.4 mmol), DMAP (0.13 g, 1.03 mmol), and then 4-monomethoxytrityl chloride (3.66 g, 11.9 mmol) were added to a stirred solution of 2′-deoxythymidine (2.50 g, 10.3 mmol) in pyridine (45 mL) at room temp. The resulting mixture was stirred overnight at room temp. The reaction mixture was cooled, and Et$_3$N (1.72 mL, 12.4 mmol) and then MsCl (0.88 mL, 11.4 mmol) were added. The mixture was stirred for 2 h at room temp., then it was filtered, the solid was washed with ethyl acetate, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOH (45 mL), and NaOH (1 M; 25 mL) was added. The mixture was heated at reflux for 1.5 h, then it was cooled to room temp. and neutralized with HCl (1 N; 10 mL). The ethanol was removed in vacuo, and the residue was extracted with CH$_2$Cl$_2$ (3× 70 mL). The combined organic extracts were washed with brine, dried with Na$_2$SO$_4$, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 2:1, v/v; 1:1, v/v; 1:2, v/v) to give **43** (2.54 g, 48%) as a pale yellow foam. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 9.85 (br. s, 1 H, NH), 7.61 (s, 1 H, 6-H), 7.48–7.46 (m, 4 H, Ar-H), 7.37–

7.20 (m, 8 H, Ar-H), 6.86–6.83 (m, 2 H, Ar-H), 6.16 (dd, J = 7.8, 1.7 Hz, 1 H, 1′-H), 4.42–4.40 (m, 1 H, 3′-H), 4.06–4.01 (m, 1 H, 4′-H), 3.78 (s, 3 H, OCH_3), 3.66–3.61 (m, 1 H, 5′-H), 3.61–3.46 (m, 1 H, 5′′-H), 2.56–2.479 (m, 1 H, 2′-H), 2.29–2.24 (m, 1 H, 2′′-H), 1.72 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4, 158.8, 151.0, 144.1, 144.0, 137.4, 135.1, 130.4, 128.4, 128.3, 128.1, 127.2, 113.4, 109.8, 87.2, 85.6, 83.5, 62.2, 55.3, 41.4, 12.5 ppm. HRMS: calcd. for $C_{30}H_{30}N_2O_6$ [M + Na]$^+$ 537.1996; found 537.1995.

3′-*O*-Phthalimido-2′-deoxythymidine (44): DIAD (0.98 g, 4.85 mmol) was added dropwise to a stirred suspension of **43** (1.85 g, 3.59 mmol), *N*-hydroxyphthalimide (0.79 g, 4.85 mmol), and PPh_3 (1.25 g, 4.75 mmol) in toluene (35 mL) at 0 °C. The reaction mixture was allowed to warm slowly to room temp. and stirred for 2 h. After the reaction was complete, the mixture was concentrated in vacuo, and the residue was dissolved in AcOH (80%; 50 mL). This solution was stirred for 3 h at room temp., then the mixture was concentrated in vacuo, coevaporating with toluene (3×). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 97:3, v/v) to give **44** (0.42 g, 28% over two steps) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.34 (br. s, 1 H, NH), 7.89 (s, 4 H, Ar-H), 7.72 (s, 1 H, 6-H), 6.39 (dd, J = 8.9, 5.6 Hz, 1 H, 1′-H), 5.20–5.14 (m, 1 H, 3′-H), 4.97–4.95 (m, 1 H, OH), 4.23–4.22 (m, 1 H, 4′-H), 3.62–3.59 (m, 2 H, 5′-H and 5′′-H), 2.46–2.26 (m, 1 H, 2′-H and 2′′-H), 1.77 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.7 (C-4), 163.6 (CO-phthalimide), 150.5 (C-2), 135.9 (C-6), 134.9 (Ar-C), 128.6 (Ar-C), 123.4 (Ar-C), 109.7 (C-5), 88.1 (C-1′), 83.7 (app. C-3′), 82.9 (app. C-4′), 61.4 (C-5′), 35.4 (C-2′), 12.3 (CH_3) ppm. HRMS: calcd. for $C_{18}H_{17}N_3O_7$ [M + Na]$^+$ 410.0959; found 410.0958.

5′-*O*-(Dibenzylphosphate)-3′-*O*-phthalimido-2′-deoxythymidine (45): Following a similar procedure to that used for the synthesis of **22**, compound **45** was obtained starting from **44** (0.40 g, 1.03 mmol), tetrazole (0.45 M in MeCN; 11.47 mL, 5.16 mmol), and dibenzyldiisopropyl phosphoramidite (0.78 mL, 2.27 mmol) in dry CH_2Cl_2 (5 mL), and H_2O_2 (30%; 0.44 mL, 5.16 mmol). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 100:0, v/v; 99:1, v/v; 98:2, v/v) to give **45** (0.62 g, 98%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.08 (br. s, 1 H, NH), 7.82–7.73 (m, 4 H, Ar-H), 7.37 (s, 1 H, 6-H), 7.31–7.30 (m, 10 H, Ar-H), 6.49 (dd, J = 8.2, 6.0 Hz, 1 H, 1′-H), 5.12–5.00 (m, 4 H, OCH_2Ph), 4.86–4.84 (m, 1 H, 3′-H), 4.53–4.52 (m, 1 H, 4′-H), 4.26–4.25 (m, 2 H, 5′-H and 5′′-H), 2.69–2.63 (m, 1 H, 2′-H), 2.10–2.02 (m, 1 H, 2′′-H), 1.80 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1 (C-4), 163.6 (CO-phthalimide), 150.3 (C-2), 135.3 (C-6), 135.2 (d, $^3J_{C,P}$ = 6.1 Hz, 1C of OCH_2Ph), 134.7 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 127.9 (Ar-C), 127.8 (Ar-C), 123.6 (Ar-C), 111.2 (C-5), 87.8 (C-1′), 84.9 (C-3′), 81.0 (d, $^3J_{C,P}$ = 8.3 Hz, C-4′), 69.6 (app t, $^2J_{C,P}$ = 5.0 Hz, OCH_2Ph), 67.0 (d, $^2J_{C,P}$ = 5.4 Hz, C-5′), 36.4 (C-2′), 12.2 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.8 ppm. HRMS: calcd. for $C_{31}H_{30}N_3O_{12}P$ [M + H]$^+$ 648.1742; found 648.1728.

3′-*O*-Amino-5′-*O*-(dibenzylphosphate)-2′-deoxythymidine (46): Methylamine (4% in H_2O; 2.73 mL, 3.52 mmol) was slowly added to a stirred solution of **45** (0.57 g, 0.88 mmol) in EtOH (15 mL) at 0 °C. The mixture was stirred at room temp. for 0.5 h, then it was filtered, and the solid was washed with CH_2Cl_2. The filtrate was concentrated in vacuo, and the crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 97:3, v/v) to give **46** (0.37 g, 81%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.91 (br. s, 1 H, NH), 7.39 (s, 1 H, 6-H), 7.34 (s, 10 H, Ar-H), 6.28 (dd, J = 8.2, 5.8 Hz, 1 H, 1′-H), 5.46 (br. s, 2 H, ONH_2), 5.12–4.99 (m, 4 H, OCH_2Ph), 4.22–4.19 (m, 4 H, 3′-H, 4′-H, 5′-H, and 5′′-H), 2.93–2.92 (m, 1 H, 2′-H), 2.40–2.34 (m, 1 H, 2′′-H), 1.81 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1 (C-4), 150.6 (C-2), 135.4 (d, $^3J_{C,P}$ = 6.3 Hz, 1C of OCH_2Ph), 135.2 (C-6), 128.8 (Ar-C), 128.6 (Ar-C), 128.0 (Ar-C), 111.3 (C-5), 84.8 (C-1′), 83.5 (C-3′), 81.5 (d, $^3J_{C,P}$ = 8.1 Hz, C-4′), 69.7–69.6 (2 d, $^2J_{C,P}$ = 5.2 Hz, 2 OCH_2Ph), 67.9 (d, $^2J_{C,P}$ = 5.6 Hz, C-5′), 36.4 (C-2′), 12.2 (-CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.5 ppm. HRMS: calcd. for $C_{31}H_{30}N_3O_{12}P$ [M + Na]$^+$ 540.1506; found 540.1501.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Glu(δ-carboxamide)-OMe]-2′-deoxythymidine (47): DCC (77.0 mg, 0.371 mmol) was added to a stirred solution of **46** (120 mg, 0.232 mmol), dipeptide **2a** (85.5 mg, 0.267 mmol), and DMAP (1.10 mg, 0.009 mmol) in a mixture of dry DMF (1.0 mL) and dry CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was allowed to warm slowly to room temp. and stirred for 24 h. All the volatiles were removed under reduced pressure, and CH_2Cl_2 (10 mL) was added to the residue. The solid residue was filtered off and washed with CH_2Cl_2. The combined organic layers were concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:2, v/v; 97:4, v/v; 94:7, v/v) to give **47** (120 mg, 63%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.65 (br. s, 1 H, NH), 10.34 (br. s, 1 H, NH), 8.20 (s, 1 H, CHO), 7.80 (d, J = 7.9 Hz, 1 H, NH-Met), 7.74 (d, J = 7.1 Hz, 1 H, NH-Glu), 7.43 (s, 1 H, 6-H), 7.34–7.28 (m, 10 H, Ar-H of OBn), 6.24 (dd, J = 8.2, 5.7 Hz, 1 H, 1′-H), 5.08–5.00 (m, 4 H, 2 OCH_2Ph), 4.74–4.70 (m, 1 H, αH-Glu), 4.63–4.62 (m, 1 H, 3′-H), 4.57–4.53 (m, 1 H, αH-Met), 4.33 (br. s, 1 H, 4′-H), 4.25–4.17 (m, 2 H, 5′-H and 5′′-H), 3.70 (s, 3 H, OCH_3), 2.60–2.54 (m, 3 H, 2′-H and γCH_2-Met), 2.32–2.04 (unresolved m, 7 H, γCH_2-Glu, βCH_2-Met, and SCH_3), 2.00–1-91 (m, 2 H, βCH_2-Glu), 1.85–1.79 (m, 4 H, 2′′-H and CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.8 (CO_2Me-Glu), 171.7 (CO-Met), 170.5 (δCO-Glu), 164.1 (C-4), 162.4 (CHO), 151.0 (C-2), 135.2 (C-6), 135.1 (d, $^3J_{C,P}$ = 6.0 Hz, 1C of OCH_2Ph), 128.8 (Ar-C), 128.6 (Ar-C), 128.0 (Ar-C), 111.7 (C-5), 84.9 (C-3′), 84.7 (C-1′), 81.0 (d, $^3J_{C,P}$ = 6.7 Hz, C-4′), 69.8–69.6 (2 d, $^2J_{C,P}$ = 5.3 Hz, 2 -OCH_2Ph), 67.6 (d, $^2J_{C,P}$ = 4.8 Hz, C-5′), 52.6 (OCH_3), 51.4 (αC-Met), 51.0 (αC-Glu), 36.0 (C-2′), 31.4 (βC-Met), 29.6 (γC-Met), 29.0 (γC-Glu), 27.2 (βC-Glu), 15.1 (SCH_3), 12.3 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.8 ppm. HRMS: calcd. for $C_{36}H_{46}N_5O_{13}PS$ [M – H]$^-$ 818.2477; found 818.2446.

3′-*O*-[*N*-For-L-Met-L-Glu(δ-carboxamide)-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (48): Compound **48** was obtained as a white solid (96 mg, 78%) according to the general procedure used for the synthesis of **19** and **33**–**40**, starting from a stirred solution of **47** (120 mg, 0.146 mmol), Et_3N (0.041 mL, 0.293 mmol), and Pd/C (10%; Degussa; 60 mg, 50% w/w) in MeOH (15 mL). ^{1}H NMR (500 MHz, D_2O): δ = 8.16 (s, 1 H, CHO), 7.98 (s, 1 H, H-6), 6.43 (dd, J = 9.1, 5.7 Hz, 1 H, 1′-H), 4.76–4.75 (m, 1 H, 3′-H), 4.60–4.57 (m, 1 H, αH-Glu), 4.47–4.45 (m, 1 H, αH-Met), 4.38 (br. s, 1 H, 4′-H), 3.97–3.96 (m, 2 H, 5′-H and 5′′-H), 3.77 (s, 3 H, OCH_3), 2.64–2.59 (m, 2 H, γCH_2-Met), 2.53–2.49 (m, 1 H, 2′-H), 2.43–2.37 (m, 1 H, 2′′-H), 2.32–2.10 (unresolved m, 7 H, γCH_2-Glu, βCH_2-Met, and SCH_3), 2.06–2.01 (m, 2 H, βCH_2-Glu), 1.95 (s, 3 H, CH_3) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 172.9 (αCO-Glu), 172.7 (CO-Met), 171.3 (δCO-Glu), 166.4 (C-4), 163.7 (CHO), 151.4 (C-2), 137.3 (C-6), 111.4 (C-5), 85.7 (C-3′), 84.3 (C-1′), 82.1 (d, $^3J_{C,P}$ = 8.7 Hz, C-4′), 63.7 (d, $^2J_{C,P}$ = 3.5 Hz, C-5′), 52.5 (OCH_3), 51.5 (αC-Met), 50.8 (αC-Glu), 34.7 (C-2′), 30.0 (βC-Met), 28.4 (γC-Met), 28.1 (γC-Glu), 25.3 (βC-

Glu), 13.5 (SCH_3), 11.2 (CH_3) ppm. ^{31}P NMR (202 MHz, [D_6]-DMSO): δ = 3.4 ppm. HRMS: calcd. for $C_{22}H_{34}N_5O_{13}PS$ [M – H]$^-$ 638.1538; found 638.1542.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′deoxythymidine (49):[53] A solution of imidazole (1.57 g, 23.08 mmol) and TBDPSCl (2.86 mL, 11.01 mmol) in dry DMF (15 mL) was added to a stirred solution of 2′-deoxythymidine (2.54 g, 10.49 mmol) in dry DMF (20 mL) at room temp. The mixture was stirred for 4 h at room temp., then it was diluted with water (350 mL), and the aqueous layer was extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 95:5, v/v) to give **49** (4.17 g, 83%) as a white solid. HRMS: calcd. for $C_{26}H_{32}N_2O_5Si$ [M + H]$^+$ 481.2153; found 481.2156.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-*O*-(methylthiomethyl)-2′-deoxythymidine (50):[41] Acetic anhydride (19.4 mL) and acetic acid (6.2 mL) were added to a stirred solution of **49** (4.08 g, 8.50 mmol) in DMSO (27.5 mL). The reaction mixture was stirred at room temp. for 48 h, then it was concentrated under reduced pressure. The residue was neutralized with saturated aq. $NaHCO_3$ (300mL), and the mixture was extracted with ethyl acetate (3 × 180 mL). The combined organic extracts were washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 4:1, v/v; 3:1, v/v; 2:1, v/v) to give **50** (3.3 g, 72%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ = 9.54 (br. s, 1 H, N*H*), 7.70–7.67 (m, 4 H, Ar-*H*), 7.47–7.38 (m, 7 H, 6-H and Ar-*H*), 6.35 (dd, *J* = 8.5, 5.7 Hz, 1 H, 1′-H), 4.66–4.56 (m, 3 H, 3′-H and OCH_2S), 4.09–4.08 (m, 1 H, 4′-H), 4.00–3.82 (m, 2 H, 5′-H and 5′′-H), 2.49–2.43 (m, 1 H, 2′-H), 2.16–2.04 (m, 4 H, SCH_3 and 2′′-H), 1.66 (s, 3 H, CH_3), 1.10 (s, 9 H, CH_3-*t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1 (C-4), 150.6 (C-2), 135.6 (Ar-C), 135.4 (Ar-C), 135.2 (C-6), 130.2 (Ar-C), 130.1 (Ar-C), 128.1 (Ar-C), 128.0 (Ar-C), 111.3 (C-5), 85.0 (C-1′), 84.8 (C-4′), 76.2 (C-3′), 73.7 (OCH_2S), 64.1 (C-5′), 37.9 (C-2′), 27.1 [$(CH_3)_3$], 19.4 (CMe_3), 13.9 (SCH_3), 12.2 (CH_3) ppm. HRMS: calcd. for $C_{28}H_{36}N_2O_5SSi$ [M + Na]$^+$ 563.2006; found 563.2009.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-*O*-(phthalimidooxymethyl)-2′deoxythymidine (51): Sulfuryl chloride (1 M solution in CH_2Cl_2; 3.32 mL, 3.32 mmol) was added to a stirred solution of **50** (1.50 g, 2.77 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was allowed to warm slowly to room temp. over 2 h, then it was concentrated under reduced pressure to give a 3′-*O*-chloromethyluridine derivative as a sticky mass, which was used without further purification.

In a separate round-bottomed flask, *N*-hydroxyphthalimide (1.81 g, 11.08 mmol) was suspended in dry CH_2Cl_2 (30 mL), and DBU (1.45 mL, 9.69 mmol) was then added. After 10 min, the red-coloured solution was added to the crude 3′-*O*-chloromethyluridine derivative, and the reaction mixture was kept stirring at room temp. for 24 h. The mixture was then diluted with CH_2Cl_2 (100 mL), and acetic acid (1 M aq.; 30 mL) was added with vigorous stirring. The aqueous layer was discarded, and the organic layer was washed with saturated aq. $NaHCO_3$ (2 × 50 mL) and brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 97:3, v/v) to give **51** (1.73 g, 95%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ = 9.35 (br. s, 1 H, N*H*), 7.84–7.69 (m, 8 H, Ar-*H*), 7.53 (s, 1 H, 6-H), 7.45–7.37 (m, 6 H, Ar-*H*), 6.34 (dd, *J* = 9.2, 5.1 Hz, 1 H, 1′-H), 5.16 (s, 2 H, OCH_2O), 5.11–5.09 (m, 3 H, 3′-H), 4.18 (br. s, 1 H, 4′-H), 4.14–4.00 (m, 2 H, 5′-H and 5′′-H), 2.56–2.50 (m, 1 H, 2′-H), 2.26–2.17 (m, 2 H, 2′′-H), 1.59 (s, 3 H, CH_3), 1.14 (s, 9 H, CH_3-*t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0 (C-4), 163.5 (CO-phthalimide), 150.6 (C-2), 135.7 (Ar-C), 135.4 (Ar-C), 135.3 (C-6), 134.6 (Ar-C), 133.2 (Ar-C), 132.4 (Ar-C), 130.2 (Ar-C), 130.1 (Ar-C), 129.0 (Ar-C), 128.1 (Ar-C), 128.0 (Ar-C), 123.7 (Ar-C), 111.3 (C-5), 97.5 (OCH_2O), 85.3 (C-1′), 84.7 (C-4′), 78.6 (C-3′), 64.6 (C-5′), 37.9 (C-2′), 27.1 [$(CH_3)_3$], 19.5 (CMe_3), 12.1 (CH_3) ppm. HRMS: calcd. for $C_{35}H_{37}N_3O_8Si$ [M + Na]$^+$ 678.2242; found 678.2258.

3′-*O*-(Phthalimidooxymethyl)-2′-deoxythymidine (52): Triethylamine trihydrofluoride (1.72 mL, 10.55 mmol) was added to a stirred solution of **51** (1.73 g, 2.64 mmol) in THF (25 mL) at room temp. The reaction mixture was stirred at room temp. for 36 h, and then it was concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 95:5, v/v) to give **52** (0.93 g, 84%) as a white solid. ^1H NMR (300 MHz, [D_6]DMSO): δ = 11.30 (s, 1 H, N*H*), 7.88 (s, 4 H, Ar-*H*), 7.78 (d, *J* = 1.0 Hz, 1 H, 6-H), 6.12 (dd, *J* = 8.9, 5.6 Hz, 1 H, 1′-H), 5.26–5.19 (m, 3 H, OCH_2O and 5′-OH), 4.80–4.78 (m, 1 H, 3′-H), 4.03–4.02 (m, 1 H, 4′-H), 3.78–3.63 (m, 2 H, 5′-H and 5′′-H), 2.38–2.32 (m, 1 H, 2′-H), 2.25–2.16 (m, 2 H, 2′′-H), 1.79 (d, *J* = 0.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 163.7 (C-4), 163.2 (CO-phthalimide), 150.4 (C-2), 135.9 (C-6), 134.9 (Ar-C), 128.5 (Ar-C), 123.4 (Ar-C), 109.5 (C-5), 97.8 (OCH_2O), 84.9 (C-1′), 83.9 (C-4′), 78.7 (C-3′), 61.5 (C-5′), 36.6 (C-2′), 12.3 (CH_3) ppm. HRMS: calcd. for $C_{19}H_{19}N_3O_8$ [M + Na]$^+$ 440.1064; found 440.1071.

5′-*O*-(Dibenzylphosphate)-3′-*O*-(phthalimidooxymethyl)-2′-deoxythymidine (53): Following a similar procedure to that used for the synthesis of **22**, compound **53** was obtained starting from **52** (0.93 g, 2.23 mmol), tetrazole (0.45 M in MeCN; 24.75 mL, 11.14 mmol), and dibenzyldiisopropyl phosphoramidite (1.52 mL, 4.46 mmol) in dry CH_2Cl_2 (10 mL), and H_2O_2 (30%; 0.96 mL, 11.14 mmol). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 100:0, v/v; 99:1, v/v; 98:2, v/v) to give **53** (1.39 g, 92%) as a colourless foam. ^1H NMR (300 MHz, $CDCl_3$): δ = 9.5 (app d, 1 H, N*H*), 7.84–7.74 (m, 4 H, phthamido Ar-*H*), 7.37 (d, *J* = 1.0 Hz, 1 H, 6-H), 7.38–7.33 (m, 10 H, benzyl Ar-*H*), 6.49 (dd, *J* = 9.1, 5.3 Hz, 1 H, 1′-H), 5.11–5.04 (m, 6 H, OCH_2Ph and OCH_2O), 4.85–4.83 (m, 1 H, 3′-H), 4.53–4.52 (m, 1 H, 4′-H), 4.55–4.49 (m, 1 H, 5′-H), 4.30–4.24 (m, 1 H, 5′′-H), 4.20–4.19 (m, 1 H, 4′-H), 2.37–2.30 (m, 1 H, 2′-H), 1.90–1.80 (m, 4 H, 2′′-H and CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.9 (C-4), 163.5 (CO-phthalimide), 150.6 (C-2), 135.6 (d, $^3J_{C,P}$ = 6.1 Hz, 1C of OCH_2Ph), 135.2 (C-6), 134.7 (Ar-C), 128.9 (Ar-C), 128.8 (Ar-C), 128.7 (Ar-C), 128.2 (Ar-C), 128.1 (Ar-C), 127.8 (Ar-C), 123.7 (Ar-C), 111.6 (C-5), 97.5 (OCH_2O), 84.7 (C-1′), 83.1 (d, $^3J_{C,P}$ = 8.3 Hz, C-4′), 78.3 (C-3′), 69.8–69.7 (2 d, $^2J_{C,P}$ = 5.4 Hz, 2 OCH_2Ph), 67.5 (d, $^2J_{C,P}$ = 5.7 Hz, C-5′), 37.2 (C-2′), 12.4 (-CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.5 ppm. HRMS: calcd. for $C_{31}H_{30}N_3O_{12}P$ [M + Na]$^+$ 570.1612; found 570.1615.

3′-*O*-(Aminooxymethyl)-5′-*O*-(dibenzylphosphate)-2′-deoxythymidine (54): Following a similar procedure to that used for the synthesis of **46**, compound **54** was obtained starting from **53** (1.3 g, 1.92 mmol), methylamine (4% in H_2O; 5.96 mL, 3.52 mmol), and EtOH (30 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 96:4, v/v) to give **54** (0.86 g, 82%) as a colourless foam. ^1H NMR (300 MHz, $CDCl_3$): δ = 9.75 (br. s, 1 H, N*H*), 7.34–7.33

(m, 11 H, 6-H and Ar-*H*), 6.25 (app t, J = 6.6 Hz, 1 H, 1′-H), 5.64 (br. s, 2 H, NH_2), 5.12–4.99 (m, 4 H, 2 OCH_2Ph), 4.78–4.72 (m, 2 H, OCH_2O), 4.31–4.26 (m, 1 H, 3′-H), 4.22–4.12 (m, 3 H, 4′-H, 5′-H, and 5′′-H), 2.41–2.33 (m, 1 H, 2′-H), 2.03–1.98 (m, 1 H, 2′′-H), 1.81 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0 (C-4), 150.5 (C-2), 135.4 (d, $^3J_{C,P}$ = 6.3 Hz, 1C of OCH_2Ph), 135.3 (C-6), 128.9 (Ar-C), 128.7 (Ar-C), 128.1 (Ar-C), 111.2 (C-5), 98.3 (OCH_2O), 84.8 (C-1′), 82.9 (d, $^3J_{C,P}$ = 7.9 Hz, C-4′), 76.4 (C-3′), 69.8–69.7 (2 d, $^2J_{C,P}$ = 5.5 Hz, 2 OCH_2Ph), 66.5 (d, $^2J_{C,P}$ = 5.5 Hz, C-5′), 38.2 (C-2′), 12.4 (-CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.4 ppm. HRMS: calcd. for $C_{25}H_{30}N_3O_9P$ [M + H]$^+$ 548.1792; found 548.1812.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Glu-(methyloxy-δ-carboxamide)-OMe]-2′-deoxythymidine (55): Following a similar procedure to that used for the synthesis of **47**, compound **55** was obtained starting from **54** (200 mg, 0.365 mmol), dipeptide **2a** (134.6 mg, 0.420 mmol), DCC (120.6 mg, 0.584 mmol), and DMAP (1.8 mg, 0.0146 mmol) in a mixture of dry DMF (2.0 mL) and dry CH_2Cl_2 (10 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 94:6, v/v) to give **55** (211.0 mg, 68%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.52 (br. s, 1 H, N*H*), 10.20 (br. s, 1 H, N*H*), 8.18 (s, 1 H, C*H*O), 7.90 (d, J = 7.0 Hz, 1 H, N*H*-Met), 7.74 (d, J = 6.7 Hz, 1 H, N*H*-Glu), 7.34–7.33 (m, 11 H, H-6 and Ar-*H* of OBn), 6.24 (app t, J = 6.5 Hz, 1 H, 1′-H), 5.13–5.00 (m, 4 H, 2 OCH_2Ph), 4.92–4.86 (m, 2 H, OCH_2O), 4.79–4.72 (m, 1 H, 3′-H), 4.54–4.52 (m, 1 H, α*H*-Glu), 4.42 (br. s, 1 H, α*H*-Met), 4.34–4.30 (m, 1 H, 5′-H), 4.22–4.16 (m, 2 H, 4′-H and 5′′-H), 3.71 (s, 3 H, OCH_3), 2.56 (t, J = 7.2 Hz, 2 H, γCH_2-Met), 2.44–2.42 (m, 1 H, 2′-H), 2.35–2.15 (m, 3 H, 2′′-H and γCH_2-Glu), 2.14–1.85 (unresolved m, 7 H, βCH_2-Met, SCH_3, and βCH_2-Glu), 1.79 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.9 (αCO-Glu), 171.8 (CO-Met), 170.0 (δCO-Glu), 164.1 (C-4), 162.1 (CHO), 150.8 (C-2), 135.3 (C-6), 135.2 (1C of OCH_2Ph), 128.9 (Ar-C), 128.7 (Ar-C), 128.0 (Ar-C), 111.4 (C-5), 98.0 (OCH_2O), 84.8 (C-1′), 82.9 (d, $^3J_{C,P}$ = 6.8 Hz, C-4′), 77.1 (C-3′; merged with $CDCl_3$), 68.9–69.8 (2 d, $^2J_{C,P}$ = 5.6 Hz, 2 OCH_2Ph), 66.7 (d, $^2J_{C,P}$ = 5.1 Hz, C-5′), 52.5 (OCH_3), 51.8 (αC-Met), 51.1 (αC-Glu), 37.7 (C-2′), 31.7 (βC-Met), 29.8 (γC-Met), 29.1 (γC-Glu), 27.3 (βC-Glu), 15.1 (SCH_3), 12.3 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.6 ppm. HRMS: calcd. for $C_{37}H_{48}N_5O_{14}PS$ [M – H]$^-$ 848.2583; found 848.2560.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Glu-(methyloxy-δ-carboxamide)-NH_2]-2′-deoxythymidine (56): Following a similar procedure to that used for the synthesis of **47**, compound **56** was obtained starting from **54** (200 mg, 0.365 mmol), dipeptide **2b** (128.3 mg, 0.420 mmol), DCC (120.6 mg, 0.584 mmol), and DMAP (1.8 mg, 0.0146 mmol) in a mixture of dry DMF (2.0 mL) and dry CH_2Cl_2 (10 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 97:3, v/v; 92:8, v/v) to give **56** (186.0 mg, 61%) as a white solid. ^{1}H NMR (300 MHz, [D$_4$]methanol): δ = 8.09 (s, 1 H, C*H*O), 7.42 (s, 1 H, 6-H), 7.31(br. s, 10 H, Ar-*H* of OBn), 6.17 (app t, J = 6.9 Hz, 1 H, 1′-H), 5.06–5.03 (m, 4 H, 2 OCH_2Ph), 4.86 (s, 2 H, OCH_2O), 4.52–4.48 (m, 2 H, 3′-H and α*H*-Glu), 4.39–4.35 (m, 1 H, α*H*-Met), 4.27–4.24 (m, 1 H, 4′-H and 5′-H), 4.19–4.15 (m, 2 H, 5′′-H), 2.58–2.50 (m, 2 H, γCH_2-Met), 2.47–2.34 (m, 1 H, 2′-H), 2.23–2.07 (m, 3 H, 2′′-H and γCH_2-Glu), 2.04–1.84 (unresolved m, 7 H, βCH_2-Met, SCH_3, and βCH_2-Glu), 1.71 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_4$]methanol): δ = 175.8 (αCO-Glu), 173.4 (CO-Met), 172.0 (δCO-Glu), 166.1 (C-4), 164.0 (CHO), 152.2 (C-2), 137.3 (C-6), 137.0 (d, $^3J_{C,P}$ = 6.2 Hz, 1C of OCH_2Ph), 129.9 (Ar-C), 129.7 (Ar-C), 129.2 (Ar-C), 112.0 (C-5), 99.1 (OCH_2O), 86.3 (C-1′), 84.3 (d, $^3J_{C,P}$ = 7.8 Hz, C-4′), 79.2 (C-3′), 71.1–71.0 (2 d, $^2J_{C,P}$ = 5.6 Hz, 2 OCH_2Ph), 68.7 (d, $^2J_{C,P}$ = 5.6 Hz, C-5′), 53.6 (αC-Met), 52.8 (αC-Glu), 38.4 (C-2′), 32.6 (βC-Met), 30.9 (γC-Met), 29.9 (γC-Glu), 28.7 (βC-Glu), 15.3 (SCH_3), 12.5 (CH_3) ppm. ^{31}P NMR (121 MHz, [D$_4$]methanol): δ = –1.1 ppm. HRMS: calcd. for $C_{36}H_{47}N_6O_{13}PS$ [M – H]$^-$ 833.2586; found 833.2567.

3′-*O*-[*N*-For-L-Met-L-Glu-(methyloxy-δ-carboxamide)-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (57): Compound **57** was obtained as a white solid (154 mg, 75%) according to the general procedure used for the synthesis of **19** and **33**–**40**, starting from a stirred solution of **55** (200 mg, 0.235 mmol), Et_3N (0.065 mL, 0.471 mmol), and Pd/C (10%; Degussa; 100 mg, 50% w/w) in MeOH (20 mL). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.51 (br. s, 1 H, N*H*), 11.24 (br. s, 1 H, N*H*), 9.03 (d, J = 8.0 Hz, 1 H, N*H*-Met), 8.94 (d, J = 6.4 Hz, 1 H, N*H*-Glu), 8.04 (s, 1 H, C*H*O), 7.80 (s, 1 H, 6-H), 6.11 (app t, J = 5.8 Hz, 1 H, 1′-H), 4.88–4.77 (m, 2 H, OCH_2O), 4.67–4.64 (m, 1 H, 3′-H), 4.49–4.45 (m, 1 H, α*H*-Glu), 4.17–4.13 (m, 1 H, α*H*-Met), 4.06–4.02 (m, 1 H, 5′-H), 3.99 (br. s, 1 H, 4′-H), 3.92–3.90 (m, 2 H, 5′′-H), 3.60 (s, 3 H, OCH_3), 2.46 (t, J = 7.9 Hz, 2 H, γCH_2-Met), 2.34–2.29 (m, 1 H, 2′-H), 2.26–2.06 (m, 3 H, 2′′-H and γCH_2-Glu), 2.04 (s, 3 H, SCH_3), 2.03–1.86 (m, 4 H, βCH_2-Glu and βCH_2-Met), 1.81 (s, 3 H, CH_3) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 172.1 (αCO-Glu), 171.6 (CO-Met), 168.7 (δCO-Glu), 164.0 (C-4), 161.2 (CHO), 150.5 (C-2), 136.2 (C-6), 109.8 (C-5), 96.2 (OCH_2O), 83.6 (C-1′), 83.2 (d, $^3J_{C,P}$ = 5.8 Hz, C-4′), 74.1 (C-3′), 62.5 (d, $^2J_{C,P}$ = 4.8 Hz, C-5′), 52.1 (αC-Met), 52.0 (OCH_3), 50.7 (αC-Glu), 36.9 (C-2′), 32.4 (βC-Met), 29.5 (γC-Met), 29.4 (γC-Glu), 27.0 (βC-Glu), 14.8 (SCH_3), 12.3 (CH_3) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = 0.0 ppm. HRMS: calcd. for $C_{23}H_{36}N_5O_{14}PS$ [M – H]$^-$ 668.1644; found 668.1656.

3′-*O*-[*N*-For-L-Met-L-Glu-(methyloxy-δ-carboxamide)-NH_2]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (58): Compound **58** was obtained as a white solid (135 mg, 73%) according to the general procedure used for the synthesis of **19** and **33**–**40**, starting from a stirred solution of **56** (180.0 mg, 0.216 mmol), Et_3N (0.06 mL, 0.431 mmol) and Pd/C (10%; Degussa; 90.0 mg, 50% w/w) in a mixture of EtOH (18 mL) and H_2O (2 mL). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 12.26 (br. s, 1 H, N*H*), 11.28 (br. s, 1 H, N*H*), 9.26 (d, J = 7.7 Hz, 1 H, N*H*-Met), 8.37 (d, J = 7.7 Hz, 1 H, N*H*-Glu), 8.07 (s, 1 H, C*H*O), 7.80 (s, 1 H, 6-H), 7.34 (s, 1 H, CONH_2-Glu), 7.04 (s, 1 H, CONH_2-Glu), 6.13 (app t, J = 6.1 Hz, 1 H, 1′-H), 4.87–4.79 (m, 2 H, OCH_2O), 4.65–4.62 (m, 1 H, 3′-H), 4.40–4.36 (m, 1 H, α*H*-Glu), 4.12–4.09 (m, 1 H, α*H*-Met), 4.02–4.00 (m, 2 H, 4′-H and 5′-H), 3.92–3.89 (m, 1 H, 5′′-H), 2.48 (t, J = 8.0 Hz, 2 H, γCH_2-Met), 2.33–2.29 (m, 1 H, 2′-H), 2.26–2.22 (m, 1 H, 2′′-H), 2.13–2.00 (unresolved m, 5 H, γCH_2-Glu and SCH_3), 1.98–1.82 (unresolved m, 7 H, βCH_2-Glu, βCH_2-Met, and CH_3) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 173.2 (αCO-Glu), 171.2 (CO-Met), 169.0 (δCO-Glu), 163.9 (C-4), 161.6 (CHO), 150.4 (C-2), 136.1 (C-6), 109.7 (C-5), 96.0 (OCH_2O), 83.6 (C-1′), 83.1 (d, $^3J_{C,P}$ = 6.0 Hz, C-4′), 74.3 (C-3′), 62.6 (d, $^2J_{C,P}$ = 4.1 Hz, C-5′), 52.5 (αC-Met), 51.2 (αC-Glu), 36.7 (C-2′), 31.7 (βC-Met), 29.6 (γC-Met), 29.5 (γC-Glu), 28.2 (βC-Glu), 14.7 (SCH_3), 12.2 (CH_3) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = 0.0 ppm. HRMS: calcd. for $C_{22}H_{35}N_6O_{13}PS$ [M – H]$^-$ 653.1647; found 653.1644.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-*O*-[*N*-For-Gly-L-Glu(γ-methyloxy ester)-L-Ala-OMe]-2′-deoxythymidine (59): Following a similar procedure to that used for the synthesis of **51**, compound **59** was obtained in two steps starting from **50** (1.69 g, 3.12 mmol) and SO_2Cl_2 (1 M in CH_2Cl_2; 3.75 mL, 3.75 mmol), followed by reaction

with tripeptide **5a** (1.24 g, 3.91 mmol) and DBU (0.61 mL, 4.06 mmol) in dry CH_2Cl_2 (50 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 96:4, v/v) to give **59** (1.87 g, 74%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.36 (s, 1 H, N*H*-Thy), 8.43 (d, J = 6.7 Hz, 1 H, N*H*-Ala), 8.21 (t, J = 5.5 Hz, 1 H, N*H*-Gly), 8.11 (d, J = 8.3 Hz, 1 H, N*H*-Glu), 8.05 (s, 1 H, C*H*O), 7.65–7.41 (m, 11 H, 6-H and Ar-*H* of TBDPS), 6.14 (app t, J = 7.0 Hz, 1 H, 1′-H), 5.32 (dd, J = 19.9, 6.6 Hz, 2 H, OCH_2O), 4.50–4.48 (m, 1 H, 3′-H), 4.40–4.32 (m, 1 H, α*H*-Glu), 4.29–4.20 (m, 1 H, α*H*-Ala), 4.04–4.01 (m, 1 H, 4′-H), 3.93–3.82 (m, 2 H, 5′-H and 5′′-H), 3.76 (d, J = 5.8 Hz, 2 H, αCH_2-Gly), 3.57 (s, 3 H, OCH_3), 2.39 (t, J = 8.1 Hz, 2 H, γCH_2-Glu), 2.34–2.25 (m, 1 H, 2′-H and 2′′-H), 1.98–1.74 (m, 2 H, βCH_2-Glu), 1.51 (s, 3 H, CH_3-Thy), 1.28 (d, J = 7.3 Hz, 3 H, CH_3-Ala), 1.02 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.8 (CO-Ala), 172.0 (δCO-Glu), 170.7 (αCO-Glu), 168.2 (CO-Gly), 163.5 (C-4), 161.4 (CHO), 150.3 (C-2), 135.4 (C-6), 135.1 (1C of Ph), 134.9 (1C of Ph), 132.7 (Ar-C), 132.3 (Ar-C), 130.1 (Ar-C), 130.0 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 109.7 (C-5), 87.7 (OCH_2O), 83.9 (C-4′), 83.6 (C-1′), 79.1 (C-3′), 63.8 (C-5′), 51.7 (OCH_3), 51.1 (αC-Glu), 47.6 (αC-Ala), 40.5 (αC-Gly), 36.8 (C-2′), 29.7 (γC-Glu), 27.2 (βC-Glu), 26.6 (*t*Bu), 18.8 (1C of *t*Bu), 16.6 (CH_3-Ala), 11.8 (CH_3-Thy) ppm. HRMS: calcd. for $C_{39}H_{51}N_5O_{12}Si$ [M – H]$^-$ 808.3230; found 808.3233.

3′-*O*-[*N*-For-Gly-L-Glu(γ-methyloxy ester)-L-Ala-OMe]-2′-deoxythymidine (60): Following a similar procedure to that used for the synthesis of **52**, compound **60** was obtained starting from **59** (0.60 g, 0.74 mmol) and $Et_3N{\cdot}3HF$ (0.483 mL, 2.96 mmol) in THF (10 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 96:4, v/v; 92:8, v/v) to give **60** (0.34 g, 80%) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.31 (s, 1 H, N*H*-Thy), 8.44 (d, J = 6.7 Hz, 1 H, N*H*-Ala), 8.21 (t, J = 5.0 Hz, 1 H, N*H*-Gly), 8.10 (d, J = 8.0 Hz, 1 H, N*H*-Glu), 8.06 (s, 1 H, C*H*O), 7.68 (s, 1 H, 6-H), 6.11 (app t, J = 7.0 Hz, 1 H, 1′-H), 5.31 (dd, J = 11.6, 6.7 Hz, 2 H, OCH_2O), 4.39–4.35 (m, 2 H, 3′-H and α*H*-Glu), 4.27–4.23 (m, 1 H, α*H*-Ala), 3.93–3.90 (m, 1 H, 4′-H), 3.77 (d, J = 6.8 Hz, 2 H, αCH_2-Gly), 3.61–3.58 (m, 5 H, 5′-H, 5′′-H, and OCH_3), 2.40 (t, J = 8.0 Hz, 2 H, γCH_2-Glu), 2.25–2.19 (m, 1 H, 2′-H and 2′′-H), 2.00–1.80 (m, 2 H, βCH_2-Glu), 1.78 (s, 3 H, CH_3-Thy), 1.29 (d, J = 7.3 Hz, 3 H, CH_3-Ala) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.8 (CO-Ala), 172.0 (δCO-Glu), 170.7 (αCO-Glu), 168.3 (CO-Gly), 163.6 (C-4), 161.4 (CHO), 150.4 (C-2), 135.9 (C-6), 109.5 (C-5), 87.6 (OCH_2O), 84.7 (C-4′), 83.6 (C-1′), 79.3 (C-3′), 61.1 (C-5′), 51.8 (OCH_3), 51.1 (αC-Glu), 47.6 (αC-Ala), 40.5 (αC-Gly), 36.9 (C-2′), 29.8 (γC-Glu), 27.2 (βC-Glu), 16.6 (CH_3-Ala), 12.2 (CH_3-Thy) ppm. HRMS: calcd. for $C_{23}H_{33}N_5O_{12}$ [M – H]$^-$ 570.2053; found 570.2054.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-Gly-L-Glu(γ-methyloxy-ester)-L-Ala-OMe]-2′-deoxythymidine (61): Following a similar procedure to that used for the synthesis of **22**, compound **61** (383 mg, 86%) was obtained starting from **60** (306 mg, 0.535 mmol) as a colourless foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.33 (s, 1 H, N*H*-Thy), 8.43 (d, J = 6.7 Hz, 1 H, N*H*-Ala), 8.20 (t, J = 5.7 Hz, 1 H, N*H*-Gly), 8.10 (d, J = 8.1 Hz, 1 H, N*H*-Glu), 8.06 (s, 1 H, C*H*O), 7.46 (d, J = 0.9 Hz, 1 H, 6-H), 7.36–7.35 (m, 10 H, Ar-*H* of OBn), 6.12 (app t, J = 7.1 Hz, 1 H, 1′-H), 5.29 (dd, J = 15.7, 6.6 Hz, 2 H, OCH_2O), 5.05 (d, J = 8.1 Hz, 4 H, 2 OCH_2Ph), 4.37–4.29 (m, 2 H, 3′-H and α*H*-Glu), 4.27–4.19 (m, 3 H, α*H*-Ala, 5′-H, and 5′′-H), 4.10–4.07 (m, 1 H, 4′-H), 3.77 (d, J = 5.8 Hz, 2 H, αCH_2-Gly), 3.58 (s, 3 H, OCH_3), 2.41 (t, J = 7.8 Hz, 2 H, γCH_2-Glu), 2.26–2.13 (m, 1 H, 2′-H and 2′′-H), 1.99–1.74 (m, 2 H, βCH_2-Glu), 1.51 (d, J = 0.9 Hz, 3 H, CH_3-Thy), 1.28 (d, J = 7.3 Hz, 3 H, CH_3-Ala) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.8 (CO-Ala), 171.9 (δCO-Glu), 170.7 (αCO-Glu), 168.3 (CO-Gly), 163.6 (C-4), 161.4 (CHO), 150.3 (C-2), 135.9 (d, $^3J_{C,P}$ = 6.8 Hz, 1C of OCH_2Ph), 135.7 (C-6), 128.5 (Ar-C), 128.4 (Ar-C), 127.8 (Ar-C), 109.9 (C-5), 87.8 (OCH_2O), 84.0 (C-1′), 82.0 (d, $^3J_{C,P}$ = 7.4 Hz, C-4′), 78.8 (C-3′), 68.7 (d, $^2J_{C,P}$ = 5.4 Hz, 2 OCH_2Ph), 66.8 (d, $^2J_{C,P}$ = 4.8 Hz, C-5′), 51.8 (OCH_3), 51.1 (αC-Glu), 47.6 (αC-Ala), 40.5 (αC-Gly), 36.5 (C-2′), 29.7 (γC-Glu), 27.2 (βC-Glu), 16.6 (CH_3-Ala), 12.0 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, [D$_6$]DMSO): δ = –0.9 ppm. HRMS: calcd. for $C_{37}H_{46}N_5O_{15}P$ [M + H]$^+$ 832.2800; found 832.2803.

3′-*O*-[*N*-For-Gly-L-Glu(γ-methyloxy ester)-L-Ala-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (62): Compound **62** was obtained as a white solid (63.6 mg, 62%) according to the general procedure used for the synthesis of **19** and **33**–**40**, starting from a stirred solution of **61** (100 mg, 0.120 mmol), $NaHCO_3$ (20.2 mg, 0.240 mmol), and 20% Pd(OH)$_2$/C (20%; 10.0 mg, 10% w/w) in EtOH/H_2O, 9:1 (10 mL). ^{1}H NMR (500 MHz, D_2O): δ = 8.14 (s, 1 H, C*H*O), 7.81 (s, 1 H, 6-H), 6.26 (app t, J = 7.2 Hz, 1 H, 1′-H), 5.38 (dd, J = 44.5, 6.9 Hz, 2 H, OCH_2O), 4.62–4.61 (m, 1 H, 3′-H), 4.39–4.34 (m, 2 H, α*H*-Glu and α*H*-Ala), 4.26–4.25 (m, 1 H, 4′-H), 3.98–3.94 (m, 4 H, αCH_2-Gly, 5′-H, and 5′′-H), 3.69 (s, 3 H, OCH_3), 2.56 (t, J = 7.4 Hz, 2 H, γCH_2-Glu), 2.46–2.34 (m, 1 H, 2′-H and 2′′-H), 2.17–1.95 (m, 2 H, βCH_2-Glu), 1.88 (d, J = 0.9 Hz, 3 H, CH_3-Thy), 1.38 (d, J = 7.3 Hz, 3 H, CH_3-Ala) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 174.5 (CO-Ala), 174.0 (δCO-Glu), 172.8 (αCO-Glu), 170.7 (CO-Gly), 166.4 (C-4), 164.6 (CHO), 151.5 (C-2), 137.4 (C-6), 111.5 (C-5), 88.3 (OCH_2O), 84.6 (C-1′), 83.9 (d, $^3J_{C,P}$ = 9.0 Hz, C-4′), 80.3 (C-3′), 64.0 (d, $^2J_{C,P}$ = 4.4 Hz, C-5′), 52.6 (OCH_3), 52.3 (αC-Glu), 48.5 (αC-Ala), 40.7 (αC-Gly), 36.8 (C-2′), 29.5 (γC-Glu), 25.6 (βC-Glu), 15.5 (CH_3-Ala), 11.4 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 1.8 ppm. HRMS: calcd. for $C_{23}H_{34}N_5O_{15}P$ [M + H]$^+$ 650.1716; found 650.1718.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-L-Met-L-Lys(Boc)-L-Lys-(Boc)-ester]-2′-deoxythymidine (63): Following a similar procedure to that used for the synthesis of **47**, compound **63** was obtained starting from **23** (250 mg, 0.498 mmol), tripeptide **4h** (378.4 mg, 0.597 mmol), DCC (164.3 mg, 0.796 mmol), and DMAP (6.1 mg, 0.050 mmol) in a mixture of dry DMF (3 mL) and dry CH_2Cl_2 (15 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH 99:1, v/v; 97:2, v/v; 94:4, v/v) to give **63** (423 mg, 76%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.51 (br. s, 1 H, N*H*), 9.31 (br. s, 1 H, N*H*), 8.22 (s, 1 H, C*H*O), 7.41 (s, 1 H, 6-H), 7.35–7.34 (m, 10 H, Ar-*H* of OBn), 6.33 (dd, J = 9.2, 5.4 Hz, 1 H, 1′-H), 5.18–5.12 (m, 1 H, 3′-H), 5.10–4.95 (m, 4 H, OCH_2Ph), 4.89–4.77 (m, 1 H, α*H*-Met), 4.73–4.34 (m, 2 H, 2 α*H*-Lys), 4.22–4.10 (m, 3 H, 4′-H, 5′-H, and 5′′-H), 3.15–3.04 (m, 4 H, 2 εCH_2-Lys), 2.56 (t, J = 6.7 Hz, 2 H, γCH_2-Met), 2.35–2.22 (m, 1 H, 2′-H), 2.13–2.07 (m, 4 H, 2′′-H and SCH_3), 1.96–1.87 (m, 2 H, βCH_2-Met), 1.82 (s, 3 H, CH_3-Thy), 1.77–1.64 (m, 4 H, 2 βCH_2-Lys), 1.54–1.31 (m, 26 H, 2 δCH_2-Lys, 2 γCH_2-Lys, and 2 CH_3-*t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.9 (CO-Lys$_{N\text{-}Ter}$), 171.7 (CO-Met), 171.3 (CO-Lys$_{C\text{-}Ter}$), 163.7 (C-4), 161.9 (CHO), 156.4 (2 OCONH), 150.9 (C-2), 135.5 (d, $^3J_{C,P}$ = 6.0 Hz, 1C of OCH_2Ph), 135.1 (C-6), 129.1 (Ar-C), 128.9 (Ar-C), 128.2 (Ar-C), 112.1 (C-5), 84.5 (C-1′), 82.6 (d, $^3J_{C,P}$ = 8.9 Hz, C-4′), 79.4 (2 C of *t*Bu), 75.7 (C-3′), 70.0 (app t, $^2J_{C,P}$ = 5.6 Hz, 2 OCH_2Ph), 67.3 (d, $^2J_{C,P}$ = 5.8 Hz, C-5′), 53.5 (2 αC-Lys), 52.8 (αC-Met), 40.1 (2 εC-Lys), 37.0 (C-2′), 31.8 (βC-Met), 31.0 (2 βC-Lys), 30.1 (γC-Met), 29.5 (2 δC-Lys), 28.6 (2 CH$_3$-*t*Bu), 22.9 (2 γC-Lys), 15.4 (SCH_3), 12.5 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, $CDCl_3$):

δ = –0.7 ppm. HRMS: calcd. for $C_{52}H_{76}N_7O_{16}PS$ $[M + H]^+$ 1118.4879; found 1118.4878.

3′-*O*-[*N*-For-L-Met-L-Lys-L-Lys-ester]-2′-deoxythymidine-5′-monophosphate TFA Salt (65): Following a similar procedure to that used for the synthesis of **41**, compound **65** was obtained as a white solid in two steps (214 mg, 62%) starting from **63** (400 mg, 0.358 mmol) and Pd/C (10%; Degussa; 200 mg, 50% w/w) in EtOH/H_2O, 10:1 (30 mL), to give crude 3′-*O*-[*N*-For-Met-L-Lys-(Boc)-L-Lys(Boc)-ester]-2′-deoxythymidine-5′-monophosphate (**64**) (335.5 mg, quantitative) as a white solid, which was immediately deprotected by treatment with H_2O (4.5 mL), thioanisole (0.056 mL, 0.476 mmol), and trifluoroacetic acid (1.5 mL). ^{1}H NMR (500 MHz, D_2O): δ = 8.11 (s, 1 H, C*H*O), 7.80 (s, 1 H, 6-H), 6.38–6.33 (m, 1 H, 1′-H), 5.47–5.44 (m, 1 H, 3′-H), 4.52–4.48 (m, 1 H, α*H*-Met), 4.46–4.33 (m, 3 H, 2 α*H*-Lys and 4′-H), 4.14–4.09 (m, 2 H, 5′-H and 5′′-H), 3.00–2.98 (m, 4 H, 2 εCH_2-Lys), 2.61–2.51 (m, 2 H, γCH_2-Met), 2.48–2.42 (m, 1 H, 2′-H and 2′′-H), 2.09 (m, 3 H, SCH_3), 2.05–1.97 (m, 2 H, βCH_2-Met), 1.91 (s, 3 H, CH_3-Thy), 1.85–1.78 (m, 4 H, 2 βCH_2-Lys), 1.72–1.66 (m, 4 H, 2 δCH_2-Lys), 1.52–1.41 (m, 4 H, 2 γCH_2-Lys) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 173.3 (CO-$Lys_{N\text{-}Ter}$), 172.7 (CO-Met), 171.7 (CO-$Lys_{C\text{-}Ter}$), 166.0 (C-4), 163.7 (CHO), 151.2 (C-2), 136.5 (C-6), 111.4 (C-5), 84.2 (C-1′), 82.7 (d, $^3J_{C,P}$ = 8.9 Hz, C-4′), 76.1 (C-3′), 64.5 (d, $^2J_{C,P}$ = 4.8 Hz, C-5′), 52.9 (αC-Lys), 52.0 (αC-Lys), 50.8 (αC-Met), 38.5 (2 εC-Lys), 35.7 (C-2′), 29.8 (βC-Met), 28.8 (2 βC-Lys), 28.4 (γC-Met), 25.5 (2 δC-Lys), 21.5 (2 γC-Lys), 13.5 (SCH_3), 11.0 (CH_3-Thy) ppm. ^{31}P NMR (202 MHz, D_2O): δ = –0.2 ppm. HRMS: calcd. for $C_{28}H_{48}N_7O_{12}PS$ $[M - H]^-$ 736.2746; found 736.2742.

3′,5′-*O*-Di(*tert*-butyldimethylsilyl)-2′-deoxythymidine (66):[51] Imidazole (3.37 g, 49.5 mmol) and then TBDMSCl (3.73 g, 24.8 mmol) were added to a stirred solution of thymidine (2.00 g, 8.26 mmol) in dry DMF, and the solution was stirred overnight at room temp. The mixture was then diluted with CH_2Cl_2, washed with water, dried with Na_2SO_4, and filtered, and the solvents were evaporated in vacuo. The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 90:10, v/v; 80:20, v/v; 70:30, v/v) to give **66** (3.03 g, 78%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.23 (br. s, 1 H, N*H*), 7.45 (s, 1 H, 6-H), 6.34–4.30 (m, 1 H, 1′-H), 4.39–4.37 (m, 1 H, 3′-H), 3.91–3.83 (m, 2 H, 5′-H and 4′-H), 3.76–3.71 (m, 1 H, 5′′-H), 2.27–2.20 (m, 1 H, 2′-H), 2.02–1.96 (m, 1 H, 2′′-H), 1.89 (s, 3 H, CH_3-Thy), 0.91 [s, 9 H, C(CH_3)$_3$], 0.97 [s, 9 H, C(CH_3)$_3$], 0.09 (s, 6 H, CH_3), 0.06 (s, 6 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.6 (C-4), 150.1 (C-2), 135.1 (C-6), 110.5 (C-5), 87.5 (C-4′), 84.5 (C-1′), 71.9 (C-3′), 62.6 (C-5′), 41.5 (C-2′), 25.6 (*t*Bu), 25.4 (*t*Bu), 18.1 (1C of *t*Bu), 17.7 (1C of *t*Bu), 12.2 (CH_3-Thy), –5.0 (CH_3), –5.2 (CH_3), –5.7 (CH_3), –5.8 (CH_3) ppm. HRMS: calcd. for $C_{22}H_{43}N_2O_5Si_2$ $[M + H]^+$ 471.2705; found 471.2706.

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxythymidine (67):[51] Compound **66** (2.39 g, 5.08 mmol) was dissolved in CH_2Cl_2 (28 mL), and the solution was cooled to 0 °C. A cooled TFA/H_2O mixture (8:1; 2.8 mL) was then added dropwise, and the reaction mixture was stirred for 4 h at 0 °C. The solution was then diluted with cooled CH_2Cl_2, washed with ice-cold water and brine, dried with Na_2SO_4, and filtered, and the solvents were evaporated in vacuo. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 95:5, v/v) to give **67** (1.07 g, 59%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.40 (br. s, 1 H, N*H*), 7.38 (d, *J* = 1.08 Hz, 1 H, 6-H), 6.13 (t, *J* = 6.7 Hz, 1 H, 1′-H), 4.48–4.43 (m, 1 H, 3′-H), 3.89–3.86 (m, 2 H, 5′-H and 5′′-H), 3.73–3.72 (m, 1 H, 4′-H), 3.07 (br. s, 1 H, O*H*), 2.29–2.27 (m, 1 H, 2′-H), 2.22–2.18 (m, 1 H, 2′′-H), 1.85 (s, 3 H, CH_3-Thy), 0.85 [s, 9 H, C(CH_3)$_3$], 0.05 (s, 6 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1 (C-4), 150.4 (C-2), 137.0 (C-6), 110.8 (C-5), 87.5 (C-4′), 86.5 (C-1′), 71.5 (C-3′), 61.8 (C-5′), 40.5 (C-2′), 25.6 (*t*Bu), 17.9 (1C of *t*Bu), 12.4 (CH_3-Thy), –4.8 (CH_3), –4.9 (CH_3) ppm. HRMS: calcd. for $C_{16}H_{29}N_2O_5Si$ $[M + H]^+$ 357.1840; found 357.1841.

5′-*O*-(Dibenzylphosphate)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxythymidine (68): Following a similar procedure to that used for the synthesis of **22**, compound **68** was obtained starting from **67** (0.47 g, 1.32 mmol), tetrazole (0.45 M in MeCN; 15 mL, 6.57 mmol), and dibenzyldiisopropyl phosphoramidite (0.95 mL, 2.87 mmol) in dry CH_2Cl_2 (10 mL), and H_2O_2 (35%; 0.57 mL, 7.01 mmol). The crude residue was purified by column chromatography on silica gel (gradient hexane/EtOAc, 90:10, v/v; 70:30, v/v; 50:50, v/v) to give **68** (0.75 g, 88%) as a colourless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.11 (br. s, 1 H, N*H*), 7.33–7.32 (m, 11 H, Ar-*H* and 6-H), 6.29 (dd, *J* = 7.5, 6.1 Hz, 1 H, 1′-H), 5.11–4.98 (m, 4 H, OCH_2Ph), 4.32–4.28 (m, 1 H, 3′-H), 4.15–4.09 (m, 2 H, 5′-H and 5′′-H), 3.95–3.93 (m, 1 H, 4′-H), 2.15 (ddd, *J* = 13.4, 6.0, 3.1 Hz, 1 H, 2′-H), 1.93–1.84 (m, 1 H, 2′′-H), 1.82 (d, *J* = 0.9 Hz, 3 H, CH_3-Thy), 0.86 [s, 9 H, C(CH_3)$_3$], 0.04 (s, 3 H, CH_3), 0.03 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.5 (C-4), 150.0 (C-2), 135.2 (1C of OCH_2Ph), 135.0 (C-6), 128.5 (Ar-C), 128.4 (Ar-C), 127.7 (Ar-C), 110.9 (C-5), 85.0 (d, $^3J_{C,P}$ = 8.0 Hz, C-4′), 84.5 (C-1′), 71.5 (C-3′), 69.4 (2 d, $^2J_{C,P}$ = 5.4 Hz, OCH_2), 66.2 (d, $^2J_{C,P}$ = 5.8 Hz, C-5′), 40.4 (C-2′), 25.3 (*t*Bu), 17.6 (1C of *t*Bu), 12.0 (CH_3-Thy), –5.0 (CH_3), –5.2 (CH_3) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –0.5 ppm. HRMS: calcd. for $C_{30}H_{42}N_2O_8PSi$ $[M + H]^+$ 617.2442; found 617.2447.

5′-*O*-(Dibenzylphosphate)-2′-deoxythymidine (23)

Method 1: TBAF (1 M in THF; 0.92 mL, 0.92 mmol) was added to a solution of **68** (570 mg, 0.92 mmol) in THF (10 mL) at 0 °C. The reaction mixture was stirred for 45 min at 0 °C, and for 15 min at room temp. The mixture was partially evaporated and then purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 96:4, v/v) to give **23** (227 mg, 49%) as a colourless foam.

Method 2: $Et_3N{\cdot}3HF$ (0.53 mL, 3.24 mmol) was added to a solution of **68** (0.80 g, 1.30 mmol) in THF (10 mL), and the solution was stirred at room temp. for 24 h. All the volatiles were removed, and the residue was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ (10% aq. solution). The organic layer was then dried with Na_2SO_4 and filtered, and the solvents were evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 98:2, v/v; 96:4, v/v) to give **23** (0.58 g, 89%) as a colourless foam.

5′-*O*-(Dibenzylphosphate)-3′-*O*-[*N*-For-Gly-L-Glu(δ-ester)-L-Phe-OMe]-2′-deoxythymidine (69): Following a similar procedure to that used for the synthesis of **47**, compound **69** was obtained starting from **23** (230 mg, 0.458 mmol), tripeptide **5b** (200.0 mg, 0.508 mmol), DCC (110.0 mg, 0.533 mmol), and DMAP (1.1 mg, 0.090 mmol) in a mixture of dry DMF (4 mL) and dry CH_2Cl_2 (4 mL). The crude residue was purified by column chromatography on silica gel (gradient CH_2Cl_2/MeOH, 99:1, v/v; 95:5, v/v; 93:7, v/v) to give **69** (230 mg, 57%) as a white foam. ^{1}H NMR (300 MHz, [D_4]methanol): δ = 8.18 (s, 1 H, C*H*O), 7.46 (s, 1 H, 6-H), 7.36 (br. s, 10 H, Ar-*H* of OBn), 7.29–7.19 (m, 5 H, Ar-*H* of Phe), 6.25 (dd, *J* = 8.6, 5.7 Hz, 1 H, 1′-H), 5.21–5.20 (m, 1 H, 3′-H), 5.13–5.09 (m, 4 H, OCH_2Ph), 4.68 (dd, *J* = 8.5, 5.7 Hz, 1 H, αC*H*-Phe), 4.68 (dd, *J* = 8.3, 5.7 Hz, 1 H, αC*H*-Glu), 4.28–4.26 (m, 2 H, 5′-H and 5′′-H), 4.19–4.18 (m, 1 H, 4′-H), 3.93 (br. s, 2 H, CH_2-Gly), 3.68

(s, 3 H, OCH_3), 3.16 (dd, J = 13.7, 5.6 Hz, 1 H, βCH_2-Phe), 3.00 (dd, J = 13.9, 8.6 Hz, 1 H, βCH_2-Phe), 2.43 (t, J = 7.1 Hz, 2 H, γCH_2-Glu), 2.37–2.33 (m, 1 H, 2′-H), 2.15–2.06 (m, 2 H, 2′′-H and βCH_2-Glu), 1.96–1.86 (m, 1 H, βCH_2-Glu), 1.77 (s, 3 H, CH_3-Thy) ppm. ^{13}C NMR (75 MHz, [D$_4$]methanol): δ = 172.0 (δCO-Glu), 171.5, 171.4, 169.1, 164.4 (C-4), 162.6 (CHO), 150.5 (C-2), 135.4 (C-6), 135.3 (d, $^3J_{C,P}$ = 6.1 Hz, 1C of OCH_2Ph), 128.6 (Ar-C), 128.2 (Ar-C), 128.1 (Ar-C), 127.8 (Ar-C), 127.5 (Ar-C), 126.2 (Ar-C), 110.4 (C-5), 84.5 (C-1′), 82.2 (d, $^3J_{C,P}$ = 7.8 Hz, C-4′), 74.1 (C-3′), 69.4 (d, $^2J_{C,P}$ = 5.7 Hz, 2 OCH_2Ph), 66.9 (d, $^2J_{C,P}$ = 5.8 Hz, C-5′), 53.5 (αC-Phe), 51.6 (αC-Glu), 51.1 (OCH_3), 40.3 (αC-Gly), 36.5 (βC-Phe), 36.0 (C-2′), 29.0 (γC-Glu), 26.4 (βC-Glu), 10.8 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, [D$_4$]methanol): δ = –1.1 ppm. HRMS: calcd. for $C_{42}H_{49}N_5O_{14}P$ [M + H]$^+$ 878.3008; found 878.3006.

3′-*O*-[*N*-For-L-Gly-L-Glu(δ-ester)-L-Phe-OMe]-2′-deoxythymidine-5′-monophosphate Triethylammonium Salt (70): Compound **70** was obtained as a white solid (160 mg, 66%) according to the general procedure used for the synthesis of **19** and **33**–**40**, starting from a stirred solution of **69** (190 mg, 0.216 mmol) and Pd/C (10%; Degussa; 120 mg, 50% w/w) in THF (20 mL). ^{1}H NMR (300 MHz, D_2O): δ = 8.15 (s, 1 H, C*H*O), 7.70 (s, 1 H, 6-H), 7.34–7.20 (m, 5 H, Ar-*H* of Phe), 6.36 (dd, J = 9.1, 5.5 Hz, 1 H, 1′-H), 5.35 (d, J = 5.2 Hz, 1 H, 3′-H), 4.68 (dd, J = 9.5, 5.5 Hz, 1 H, αC*H*-Phe), 4.29–4.24 (m, 2 H, αC*H*-Glu and 4′-H), 4.93–3.92 (m, 4 H, αCH_2-Gly, 5′-H, and 5′′-H), 3.70 (s, 3 H, OCH_3), 3.25–3.18 (m, 1 H, βCH_2-Phe), 2.99–2.91 (m, 1 H, βCH_2-Phe), 2.42–2.88 (m, 4 H, γCH_2-Glu, 2′-H, and 2′′-H), 1.98–1.82 (m, 2 H, βCH_2-Glu), 1.86 (s, 3 H, CH_3-Thy) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 173.6, 172.1, 170.4, 169.1, 164.4 (CHO), 163.8 (C-4), 153.6 (C-2), 137.4 (1C of Ph), 137.1 (C-6), 129.0 (Ar-C), 128.3 (Ar-C), 126.5 (Ar-C), 111.5 (C-5), 85.6 (d, $^3J_{C,P}$ = 8.0 Hz, C-4′), 84.5 (C-1′), 71.0 (C-3′), 63.4 (d, $^2J_{C,P}$ = 4.06 Hz, C-5′), 53.7 (αC-Phe), 55.8 (αC-Glu), 53.5 (OCH_3), 41.6 (αC-Gly), 38.0 (βC-Phe), 37.4 (C-2′), 28.6 (γC-Glu), 26.7 (βC-Glu), 11.7 (CH_3-Thy) ppm. ^{31}P NMR (121 MHz, D_2O): δ = –3.7 ppm. HRMS: calcd. for $C_{28}H_{35}N_5O_{14}P$ [M – H]$^-$ 696.1923; found 696.1926.

Supporting Information (see footnote on the first page of this article): Copies of ^{1}H, ^{13}C and ^{31}P NMR spectra.

Acknowledgments

The authors thank P. Marliere for helpful discussions. The research leading to these results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013), ERC Grant agreement number ERC-2012-ADG_20120216/ 320683.

[1] V. B. Pinheiro, P. Holliger, *Curr. Opin. Chem. Biol.* **2012**, *16*, 245–252.
[2] J. C. Chaput, H. Yu, S. Zhang, *Chem. Biol.* **2012**, *19*, 1360–1371.
[3] P. Herdewijn, P. Marliere, *Chem. Biodiversity* **2009**, *6*, 791–808.
[4] V. B. Pinheiro, A. I. Taylor, C. Cozens, M. Abramov, M. Renders, S. Zhang, J. C. Chaput, J. Wengel, S.-Y. Peak-Chew, S. H. McLaughlin, P. Herdewijn, P. Holliger, *Science* **2012**, *336*, 341–344.
[5] V. Pezo, F. W. Liu, M. Abramov, M. Froeyen, P. Herdewijn, P. Marliere, *Angew. Chem. Int. Ed.* **2013**, *52*, 8139–8143; *Angew. Chem.* **2013**, *52*, 8139–8143.
[6] J. W. Payne, N. J. Marshall, in: *Microbial Transport Systems* (Ed.: G. Winkelmann), Wiley-VCH, Weinheim, Germany, **2002**, p. 139–164.
[7] T. E. Fickel, C. Gilvarg, *Nature New Biol.* **1973**, *241*, 161–163.
[8] S. M. Hammond, A. Claesson, A. M. Jansson, L. G. Larsson, B. G. Pring, C. M. Town, B. Ekstrom, *Nature* **1987**, *327*, 730–732.
[9] F. J. M. Detmers, F. C. Lanfermeijer, B. Poolman, *Res. Microbiol.* **2001**, *152*, 245–258.
[10] C. F. Higgins, *Res. Microbiol.* **2001**, *152*, 205–210.
[11] J. R. Tame, G. N. Murshudov, E. J. Dodson, T. K. Neil, G. G. Dodson, C. F. Higgins, A. J. Wilkinson, *Science* **1994**, *264*, 1578–1581.
[12] S. H. Sleigh, J. R. Tame, E. J. Dodson, A. J. Wilkinson, *Biochemistry* **1997**, *36*, 9747–9758.
[13] C. A. Guyer, D. G. Morgan, J. V. Staros, *J. Bacteriol.* **1986**, *168*, 775–779.
[14] M. K. Doeven, R. Abele, R. Tampe, B. Poolman, *J. Biol. Chem.* **2004**, *279*, 32301–32307.
[15] M. M. Klepsch, M. Kovermann, C. Low, J. Balbach, H. P. Permentier, F. Fusetti, J. W. de Gier, D. J. Slotboom, R. P. Berntsson, *J. Mol. Biol.* **2011**, *414*, 75–85.
[16] A. Marchand, D. Marchand, R. Busson, P. Marliere, P. Herdewijn, *Chem. Biodiversity* **2007**, *4*, 1450–1465.
[17] Y. J. Hu, Y. Wei, Y. Zhou, P. T. Rajagopalan, D. Pei, *Biochemistry* **1999**, *38*, 643–650.
[18] D. Chandu, D. Nandi, *Microbiology* **2003**, *149*, 3437–3447.
[19] K. Lu, Q. P. Duan, L. Ma, D. X. Zhao, *Bioconjugate Chem.* **2010**, *21*, 187–202.
[20] S.-Y. Han, Y.-A. Kim, *Tetrahedron* **2004**, *60*, 2447–2467.
[21] Y. Masui, N. Chino, S. Sakakibara, *Bull. Chem. Soc. Jpn.* **1980**, *53*, 464–468.
[22] J.-P. Mazaleyrat, J. Xie, M. Wakselman, *Tetrahedron Lett.* **1992**, *33*, 4301–4302.
[23] M. E. Jung, A. Toyota, *J. Org. Chem.* **2001**, *66*, 2624–2635.
[24] H. W. Yang, J. W. Yi, E.-K. Bang, E. M. Jeon, B. H. Kim, *Org. Biomol. Chem.* **2011**, *9*, 291–296.
[25] T. Kline, S. R. Jackson, W. Deng, C. L. M. J. Verlinde, S. I. Miller, *Nucleosides Nucleotides Nucleic Acids* **2008**, *27*, 1282–1300.
[26] H. A. Vaccaro, Z. Zhao, J. W. Clader, L. Song, G. Terracina, L. Zhang, D. A. Pissarnitski, *J. Comb. Chem.* **2007**, *10*, 56–62.
[27] A. Semenyuk, A. Földesi, T. Johansson, C. Estmer-Nilsson, P. Blomgren, M. Brännvall, L. A. Kirsebom, M. Kwiatkowski, *J. Am. Chem. Soc.* **2006**, *128*, 12356–12357.
[28] I. Okamoto, K. Seio, M. Sekine, *Tetrahedron Lett.* **2006**, *47*, 583–585.
[29] M. Saady, L. Lebeau, C. Mioskowski, *Tetrahedron Lett.* **1995**, *36*, 2239–2242.
[30] K. C. Fylaktakidou, C. D. Duarte, A. E. Koumbis, C. Nicolau, J. M. Lehn, *ChemMedChem* **2011**, *6*, 153–168.
[31] P. Narayanasamy, D. C. Crick, *Heterocycles* **2008**, *76*, 243–247.
[32] J.-i. Matsuzaki, H. Hotoda, M. Sekine, T. Hata, S. Higuchi, Y. Nishimura, M. Tsuboi, *Tetrahedron* **1986**, *42*, 501–513.
[33] G. Dufner, R. Schwörer, B. Müller, R. R. Schmidt, *Eur. J. Org. Chem.* **2000**, 1467–1482.
[34] M. Leuck, K. E. Vagle, J. Shawn Roach, A. Wolter, *Tetrahedron Lett.* **2004**, *45*, 321–324.
[35] K. Burgess, R. A. Gibbs, M. L. Metzker, R. Raghavachari, *J. Chem. Soc., Chem. Commun.* **1994**, 915–916.
[36] D. Hutter, M.-J. Kim, N. Karalkar, N. A. Leal, F. Chen, E. Guggenheim, V. Visalakshi, J. Olejnik, S. Gordon, S. A. Benner, *Nucleosides Nucleotides Nucleic Acids* **2010**, *29*, 879–895.
[37] J. Guo, N. Xu, Z. Li, S. Zhang, J. Wu, D. H. Kim, M. Sano Marma, Q. Meng, H. Cao, X. Li, S. Shi, L. Yu, S. Kalachikov, J. J. Russo, N. J. Turro, J. Ju, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9145–9150.
[38] J. P. Horwitz, J. Chua, J. A. Urbanski, M. Noel, *J. Org. Chem.* **1963**, *28*, 942–944.
[39] J. Cieslak, A. Grajkowski, C. Ausin, A. Gapeev, S. L. Beaucage, *Nucleic Acids Res.* **2012**, *40*, 2312–2329.

[40] T. W. Johnson, S. P. Tanis, S. L. Butler, D. Dalvie, D. M. DeLisle, K. R. Dress, E. J. Flahive, Q. Hu, J. E. Kuehler, A. Kuki, W. Liu, G. A. McClellan, Q. Peng, M. B. Plewe, P. F. Richardson, G. L. Smith, J. Solowiej, K. T. Tran, H. Wang, X. Yu, J. Zhang, H. Zhu, *J. Med. Chem.* **2011**, *54*, 3393–3417.
[41] E. Rozners, D. Katkevica, R. Stromberg, *ChemBioChem* **2007**, *8*, 537–545.
[42] J. E. Gallant, S. Deresinski, *Clin. Infect. Dis.* **2003**, *37*, 944–950.
[43] C. Lu, Y. Jia, J. Yang, Y. Song, W. Liu, Y. Ding, X. Sun, A. Wen, *Clin. Drug Invest.* **2012**, *32*, 333–338.
[44] N. Parey, C. Baraguey, J. J. Vasseur, F. Debart, *Org. Lett.* **2006**, *8*, 3869–3872.
[45] R. Johnsson, J. G. Lackey, J. J. Bogojeski, M. J. Damha, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3721–3725.
[46] A. R. Martin, T. Lavergne, J.-J. Vasseur, F. Debart, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4046–4049.
[47] P. J. L. M. Quaedflieg, C. M. Timmers, V. E. Kal, G. A. van der Marel, E. Kuyl-Yeheskiely, J. H. van Boom, *Tetrahedron Lett.* **1992**, *33*, 3081–3084.
[48] J. G. Lackey, D. Mitra, M. M. Somoza, F. Cerrina, M. J. Damha, *J. Am. Chem. Soc.* **2009**, *131*, 8496–8502.
[49] E. Kiuru, M. Ora, L. Beigelman, L. Blatt, H. Lönnberg, *Chem. Biodiversity* **2011**, *8*, 266–286.
[50] W. Shen, J.-S. Kim, P. E. Kish, J. Zhang, S. Mitchell, B. G. Gentry, J. M. Breitenbach, J. C. Drach, J. Hilfinger, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 792–796.
[51] M. G. Friedel, J. C. Pieck, J. Klages, C. Dauth, H. Kessler, T. Carell, *Chem. Eur. J.* **2006**, *12*, 6081–6094.
[52] K. S. Ghosh, J. Debnath, P. Dutta, B. K. Sahoo, S. Dasgupta, *Bioorg. Med. Chem.* **2008**, *16*, 2819–2828.
[53] H. Cui, L. M. Ruiz-Perez, D. Gonzalez-Pacanowska, I. H. Gilbert, *Bioorg. Med. Chem.* **2010**, *18*, 7302–7309.

Received: November 29, 2013
Published Online: February 12, 2014

