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First Synthesis of 2′-Deoxyfluoropuromycin Analogues: Experimental Insight into the Mechanism of the Staudinger Reaction

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Abstract: The N^6,N^6-dedimethyl-2′-deoxyfluoro analogue of puromycin (= 3′-deoxy-N^6,N^6-dimethyl-3′-[*O*-methyltyrosylamido]adenosine), its 2′,3′-regioisomer and a 3′-cytidyl-5′-(2′-deoxyfluoro)puromycyl dinucleotide analogue were synthesized following an approach involving i) the diastereospecific nitrite-assisted formation of a *lyxo* nucleosidic 2′,3′-epoxide from an adenosine-2′,3′-ditriflate derivative in a biphasic solvent mixture; ii) the regio- and stereoselective epoxide ring opening with sodium azide under mildly acidic aqueous conditions, iii) the stereospecific introduction of the fluor atom using DAST and iv) the reaction between the nucleosidyl or dinucleotidyl azide and an active ester of the N-protected amino acid using highly efficient solution conditions for the Staudinger–Vilarrasa coupling, to obtain the corresponding carboxamide directly from the in situ formed iminophosphorane. This coupling reaction furnished sterically quite demanding amides in 94% isolated yields under very mild conditions and should therefore be of a more general value. Under certain reaction conditions we isolated (amino)acyltriazene derivatives from which dinitrogen was not eliminated. These secondary products are trapped and stabilized witnesses of the first intermediate of the Staudinger reaction, the phosphatriazenes (phosphazides, triazaphosphadienes) which usually eliminate dinitrogen in situ and rapidly rearrange into iminophosphoranes, unless they are derived from conjugated or sterically bulky azides and phosphines. The acyltriazenes could either be thermally decomposed or converted to the corresponding *N*-alkyl carboxamides through proton-assisted elimination of dinitrogen. All compounds were carefully characterized through MS spectrometry, ^{1}H, ^{19}F, ^{31}P and ^{13}C NMR spectroscopy.

Keywords: antibiotics · antitumor agents · azides · nucleotides · Staudinger Vilarrasa · triazenes

Introduction

The ribosome is the macromolecular machine responsible for protein synthesis in all cells. The peptidyl transferase active site of the ribosome has two substrate binding sites and utilizes two tRNAs as the reaction substrates: an aminoacyl-tRNA bound to the A-site and a peptidyl-tRNA bound to the P-site. The peptidyl transfer reaction involves aminolysis of the ester bond linking the peptide to the 3′-terminal oxygen atom of the P-site bound tRNA by the α-amino group of the A-site bound aminoacyl-tRNA (the α-amino nucleophile attacks one face of the planar ester group). This attack was thought to yield an oxyanion containing a tetrahedral carbon intermediate which would resolve to the products,[1] a deacylated P-site bound tRNA and an A-site bound peptidyl-tRNA with a peptide chain elongated by one amino acid. After translocation of the elongated peptidyl-tRNA into the P-site with the help of a translocation factor·GDP complex, new aminoacyl-tRNAs—each in conjunction with an elongation factor·GTP complex—can test the new codon presented at the decoding site. The cognate aminoacyl-tRNA is detached from the elongation factor through GTP-to-GDP hydrolysis and processed, thus, protein synthesis continues.

The tRNA substrates are aligned in the active site by base pairing between the CCA sequence at the 3′-end of each tRNA and complementary sequences within the A and P-sites of the rRNA that make up the peptidyl transferase center.[2] The ribosome can also catalyze amide-bond forma-

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tion using the minimal A-site substrate puromycin, a nucleoside antibiotic comprised of 3′-amino-3′-deoxy-N^6,N^6-dimethyladenosine coupled to the amino acid *O*-methyl-L-tyrosine (3′-deoxy-N^6,N^6-dimethyl-3′-[*O*-methyltyrosylamido]adenosine, **1**).[3] The antitumor antibiotic puromycin inhibits protein synthesis as a consequence of its striking resemblance to the aminoacyladenyl 3′-terminus of aminoacyl-tRNA. It has been demonstrated that puromycin competes with aminoacyl-tRNA at the A site and subsequently interacts with the peptidyl-tRNA at the P site causing premature release of the polypeptide chains from the ribosome.[4] For this reason, puromycin has long since been used in the investigation of the peptidyl transfer reaction.[5] Today puromycin is broadly used as a tool for molecular biologists.

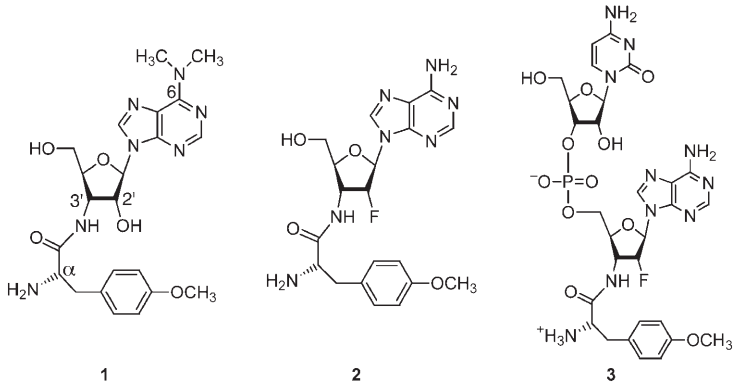


Figure 1. Natural puromycin and its 2′-deoxyfluorinated analogues.

The catalytic mechanism of the peptidyl transfer reaction is an area of ongoing research interest both for its medicinal and for its evolutionary relevance. Evolutionarily, RNA catalysis of peptide-bond formation is the reaction required to bridge between a world dominated by RNA catalysts and the one of modern cells dominated by protein catalysts.[6] Medicinally, the peptidyl transferase center with its surrounding substrate and product channels is the binding site for several naturally and synthetically derived antibiotics.[7,8]

Microbial resistance to antibiotics is growing and spreading rapidly. Thus, new approaches for the development of novel antibiotics are clearly needed and, since about one half of the currently used antibiotics target the ribosome—mostly the large subunit—structure-based drug design using the large subunit structure should be useful. The use of the structural information has greatly increased the speed with which new potential drug candidates can be developed. Furthermore, structural variation of puromycin to obtain more active analogues may be extremely useful in elucidating various aspects of protein synthesis. For this purpose, one of our priorities is to synthesize new and powerful antibiotics that will be capable of supplementing or replacing those antibiotics that become ineffective due to bacterial resistance mutations.

Numerous antibiotics have been shown to bind to bacterial and/or eucaryotic ribosomes and inhibit protein synthesis but only puromycin is a pure A-site binder that inhibits protein synthesis irreversibly and produces truncated, dysfunctional bacterial C-terminal puromycyl peptides which are likely to boost an immune response in the infected host organism. Therefore a comparison of puromycin analogues **2** and **3** (Figure 1) and other analogues[9] with natural puromycin in assays on bacterial ribosomes will be carried out. Specifically, the ability of the puromycin analogues to serve as peptide acceptors during peptidyl transfer, to inhibit protein synthesis, and kinetic parameters for the interaction of these analogues with procaryotic P-site substrates will be reported in the future.

In the puromycin analogues **2** and **3** the 2′-hydroxyl group of both natural puromycin and the peptide accepting 3′-terminal A76 residue of aminoacyl-tRNA, a function known to serve as a hydrogen acceptor of the imino hydrogen atom of the ribosomal A-site uridylate residue U2620,[10] is replaced by a close to isosteric and perhaps weakly hydrogen bonding fluor atom.[11] The 2′-fluorine atom allows for a qualitatively undisturbed peptidyl transfer when present at the 3′-terminal adenylate of an A site-bound aminoacyl-tRNA.[12] Its high electronegativity may have a favorable impact on the ribofuranose pucker, since 2′-F is likely to force, through the stereoelectronic *gauche* effect stronger than the one of 2′-OH, a more defined orientation of the vicinal 3′-substituent bearing the reactive nucleophile (Figure 1), the more so, as its α-amino group accumulates a partial positive charge during peptidyl transfer before it deprotonates, which may to some extent deplete electron density on N3′ in the first transition state and thus further reinforce the stereoelectronic *gauche* effect.[13]

Puromycin is known to carry out a much faster peptidyl transfer reaction when it is present as a dinucleotide where the 5′-terminus is a cytidylate that mimics C75 of a transfer RNA.[14] The cytidine residue in **3** is expected to stabilize the analogue's interaction with the ribosome through base pairing with the complementary A site residue G2588, as is the case for C75 of aminoacyl-tRNA.[10]

The new fluorinated puromycin analogues **2** and **3** (Figure 1) are synthesized in seven and thirteen steps, respectively, from adenosine (**4**). The synthetic route for the preparation of **2** and **3** is outlined in Schemes 1 and 3.

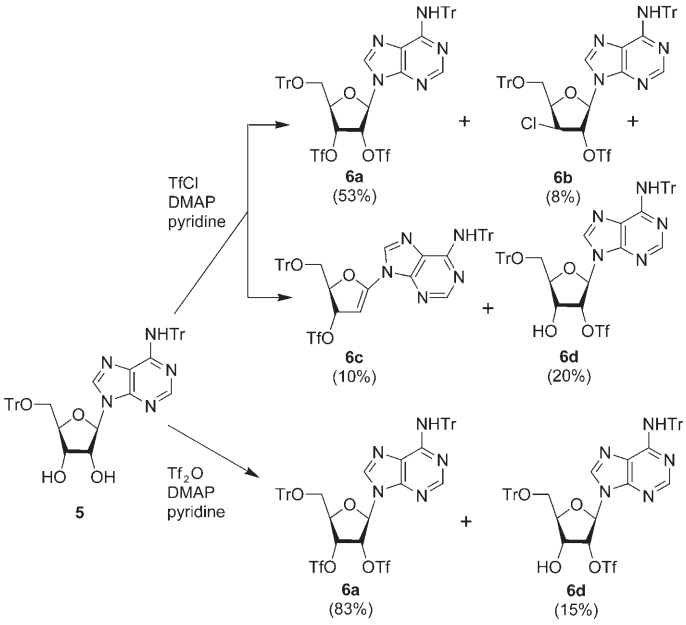
Results

The synthetic pathway (Scheme 1) is evident and conceptually similar to published procedures[15a,b] but each step was re-evaluated and changed so as to render the synthesis more efficient and practical. It begins with the synthesis of **5**, a protected form of adenosine (**4**).

The tritylation of **4** furnished at most 34% of the desired ditritylated compound **5**.[15c] The first challenge was to efficiently obtain diastereoisomerically pure *lyxo*-epoxide **7** (Scheme 1) from an activated form of diol **5**,[15a] best through an efficient synthesis of ditriflate **6a**.[15d] Our first attempts with the highly reactive triflic chloride and 4-dimethylaminopyridine (DMAP)—found to be indispensable[15e]—produced in significant yields chlorinated (**6b**), eliminated (**6c**) and monotriflated (**6d**) side products (Scheme 2) which were identified by ^{1}H and ^{19}F NMR spectra (cf. Supporting Information),[16] as well as through ESI MS spectra (not shown). Triflic anhydride and DMAP produced **6a** in 83.7% yield along with 15% isolated **6d** that were triflated to **6a** in a subsequent batch. Both **6a** and **6d** proved quite stable and could be readily worked up and purified by chromatography over silica gel without detectable elimination or hydrolysis.

In our hands, rather than from a 2′,3′-*O*,*O*-dimesylate and sodium hydroxide in ethanol,[15a,b,17] *lyxo*-epoxide **7** was more reliably and cleanly obtained from ditriflate **6a** using tetrabutylammonium nitrite in a biphasic water/toluene mixture.[15d] The initially formed mononitrite–monotriflic diester intermediate, where the nitrite bearing carbon configuration had been inversed (major regioisomer: probably 3′-*O*-nitrite-2′-*O*-triflate),[16] hydrolyzed to the corresponding monohydroxy-monotriflic ester intermediate—rather than reacting to the doubly inverted vicinal dinitrite—and subsequently ring-closed in situ to **7**.

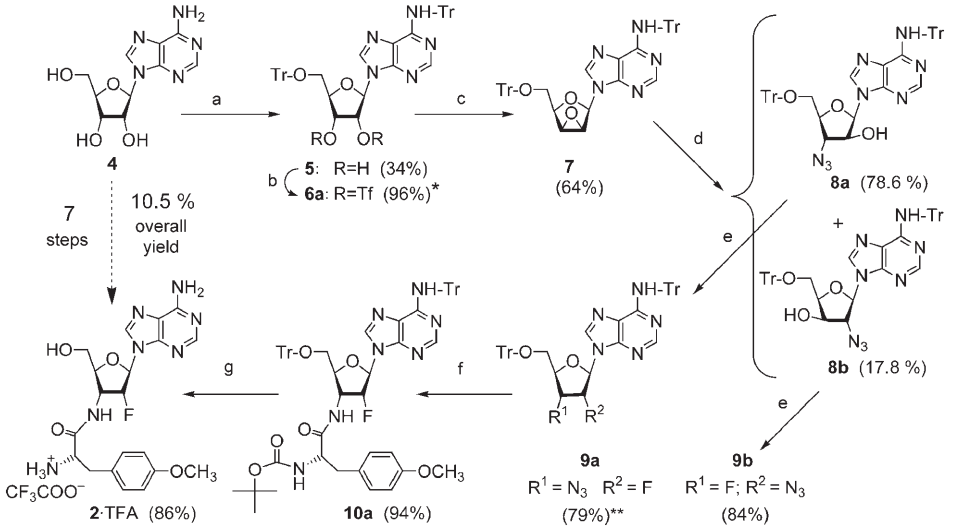
Epoxides are most often opened by nucleophiles such as the azide anion under anhydrous neutral or slightly basic conditions using well soluble lithium azide in anhydrous DMF, but rather weak regioselectivities have been observed.[15a] Higher regioselectivities were obtained with sodium azide in refluxing acetone, but only after a much prolonged reaction time (20 h).[17] We obtained the desired regioisomer **8a** in higher yields after only 60–90 minutes reaction time using soluble sodium azide in aqueous DMF in the presence of ammonium chloride.[18] The combined isolated yield for **8a** and **8b** was 93–96% and the ring opening selectivity better than 4:1 in favor of the desired regioisomer.



Scheme 2. Reactivity difference between TfCl and Tf_2O in the presence of DMAP.

The isomers could be readily separated through chromatography over silica gel and converted using DAST (Et_2NSF_3) to the corresponding deoxyfluoro derivates **9a** and **9b**, respectively, under inversion of the configuration of the alcohol function.[19] The best isolated yields were obtained when the formation of the 2′-*O*-SF_2NEt_2 intermediate at ambient temperature clearly preceded the nucleophilic substitution by F^- at 80°C.

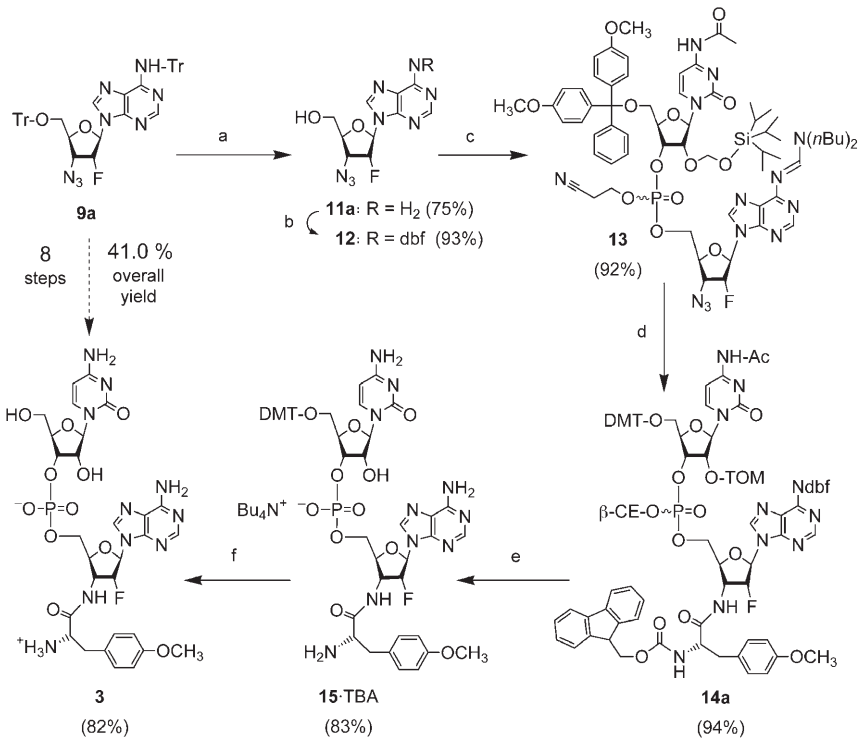
We attached the amino acid to the nucleoside by means of a direct coupling of azide **9a** to an OBt active ester of *N*-Boc-*O*-methyltyrosine in 94% yield under the recently published Staudinger–Vilarrasa coupling conditions as developed for the synthesis of non-fluorinated puromycin ana-



Scheme 1. Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-[*O*-methyltyrosylamido]adenosine (**2**). a) Tr-Cl, DMAP, Py, 80°C/4 h 40 min; b) Tf_2O, Py, DMAP, 0°C/30 min, then RT/3 h; c) $Bu_4N^+NO_2^-$, MePh/H_2O, RT/40 h; d) NaN_3, NH_4Cl, DMF/H_2O, reflux (100°C)/1 h 30 min; silica gel chromatography; e) DAST, Py, Tol, RT/30 min, then 80°C/45 min; f) Boc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0°C/30 min, then RT/overnight; g) CF_3COOH, CH_2ClCH_2Cl, RT/4 h. * This combined yield was obtained when monotriflate **6d** (see text, Experimental Section and Supporting Information) was reused for the synthesis of **6a**. ** Yield with respect to consumed **8a** (=applied − recovered).

logues.[9] Compound **10a** was deprotected with TFA (CF_3COOH)[15c] in dichloroethane to give **2** as the TFA salt in 10.5% overall yield from adenosine (**4**).

The isolation of the pure and highly water soluble target compound **2**·TFA necessitates the following procedure. The deprotection reaction must be quenched with an excess methanol to avoid partial retritylation. The resulting mixture is concentrated under reduced pressure to a volume that can be directly applied on a silica gel column. Chromatography yields the target compound as a mixture of α-amine and α-ammonium salt as determined by ^{1}H-decoupled ^{19}F NMR (integral ratio between the signals at −76.9 ppm for TFA^- and −196.5 ppm for 2′-F of compound **2** ≈1.34:1). To enhance the solubility in water and to isolate a homogenous salt form of the target compound, this solution (about 10 mM) was carefully acidified with more TFA until ^{19}F NMR showed complete formation of the **2**·TFA salt (^{19}F NMR signal integral ratio TFA^-/2′-F 3:1) which consistently resulted in a pH of 3.4. Such a solution can be safely lyophilized to furnish **2**·TFA as a well soluble white powder.



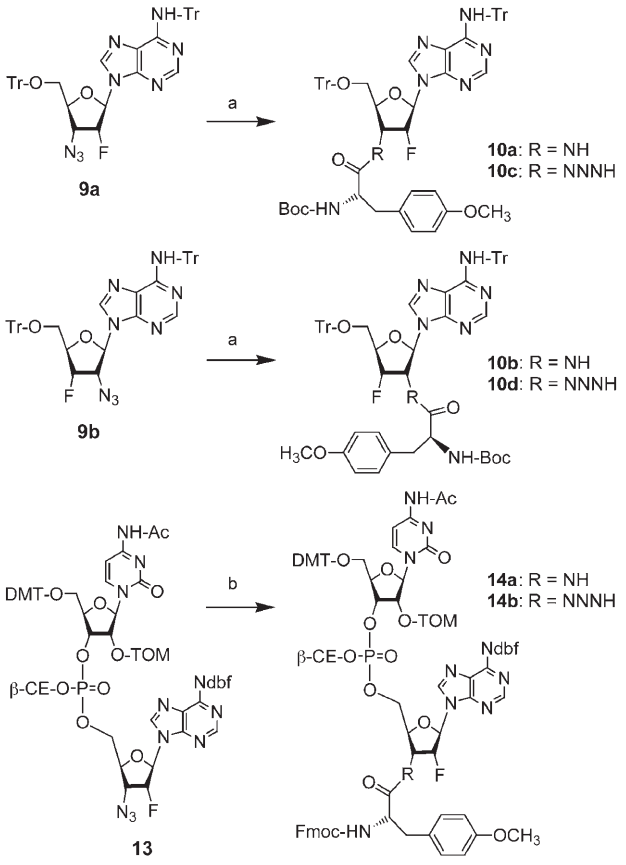
Scheme 3. Synthesis of cytid-3′-yl-5′-[2′,3′-dideoxy-2′-fluoro-3′-(*O*-methyltyrosyl)amido]adenylate (**3**). a) CF_3COOH, CH_2ClCH_2Cl, RT/2 h; b) *N*,*N*-di(*n*-butyl)formamide dimethyl acetal, MeOH, RT/15 min; c) 1) ethylthiotetrazole, N^4-acetyl-5′-*O*-dimethoxytrityl-2′-*O*-triisopropylsilyloxymethylcytid-3′-yl-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, dry CH_3CN, RT/20 min, 2) 0.2 M I_2/THF/pyridine/H_2O, RT; d) Fmoc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0 °C/30 min, then RT/overnight; e) 1) 33% CH_3NH_2/EtOH, RT/35 min, 2) TBAF, THF, RT/40 min; f) 80% AcOH/H_2O, RT/20 min.

The synthesis of dinucleotide analogue **3** (Scheme 3) starts from intermediate **9a** which was de- and reprotected,[15c,20] to give first **11a** then **12** (**9b** was deprotected to give **11b**, cf. Experimental Section and Supporting Information). Compound **12** was coupled to a commercial cytidine phosphoramidite precursor according to standard procedures.[21] Azidodinucleotide **13** was an equally efficient coupling partner under the Staudinger–Vilarrasa conditions (94% yield). Complete deprotection of **14a** in three steps furnished the target compound **3** with 5.3% overall yield from **4**.

During the optimization of the Staudinger–Vilarrasa coupling reaction conditions a slight modification of the protocol—holding the reaction temperature at 0 °C all times—was sufficient to allow for the appearance of a secondary product of very similar polarity (similar R_f values on TLC plates) and showing reasonably similar ^{1}H but distinct ^{19}F NMR spectra from the ones of the corresponding and desired amides and a molecular mass of +28 Da higher than the target compounds. Apparently, trapping the initially formed phosphatriazene with the activated amino acid had become possible before it eliminated dinitrogen (Scheme 4). We detected the acyltriazene derivatives in varied but substantial amounts, as determined by ESI mass (Table 1), ^{1}H and ^{19}F NMR spectra (Figures 2 and 3, respectively) after chromatographic purification of the reaction mixtures but no separation of the acyltriazene from the amide. When tributylphosphine was first mixed with **9b** separately at 0 °C followed by the addition to a solution containing the activated amino acid at 0 °C, we obtained after 48 h reaction time (instead of the usual 16 h) even more of the acyltriazene **10d** (Table 1, entry 3). We obtained the highest acyltriazene-to-amide ratio, approximately 1:1, with the coupling at 0 °C on dinucleotide **13** (Table 1, entry 4).

The ^{1}H and ^{19}F NMR spectra of the mixtures **10a** + **10c** (Table 1, entry 1), **10b** + **10d** (Table 1, entry 3) and **14a** + **14b** (Table 1, entry 4) confirmed the assumption of comparable volatility of the molecular ions of the amides and acyltriazenes—although some acyltriazene contents may appear slightly underestimated in the ESI mass spectra—and revealed useful additional informations about the nature of the acyltriazenes (Figures 2 and 3).

In all three mixtures the presence of the acyltriazene in deuterochloroform gave rise to several more or less broadened ^{1}H resonances (Figure 2) at low (9.5–10.5 ppm for **10c**, **10d** and **14b**) and very low fields (13–14 ppm for **14b**). The corresponding hydrogen atoms exchanged rapidly against



Scheme 4. Formation of amides **10a**, **10b** and **14a** and acyltriazenes **10c**, **10d** and **14b**: a) Boc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0°C overnight; b) Fmoc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0°C overnight.

Table 1. Ratio of amides **10a**, **10b** and **14a** versus acyltriazenes **10c**, **10d** and **14b**, respectively, under conditions that favor the enrichment of acyltriazenes.

Entry	Compounds	Reagents	Ratio[b] amide/acyltriazene
1	**9a**	Boc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0°C, overnight[a]	91:9 **10a**/**10c**
2	**9b**	Boc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0°C, overnight[a]	87–90:13–10[c] **10b**/**10d**
3	**9b** + P(*n*Bu)$_3$ (0°C)	Boc-L-Tyr(Me)-OH, HOBt, DIC, 0°C, overnight	78:22 **10b**/**10d**
4	**13**	Fmoc-L-Tyr(Me)-OH, HOBt, DIC, P(*n*Bu)$_3$, 0°C, overnight[a]	53:47 **14a**/**14b**

[a] P(*n*Bu)$_3$ was added to the reaction mixture as the last reagent, procedure as described in the Experimental Section. [b] The ratios were determined through the ratio of the ESI$^+$ mass peak intensities of the corresponding amide and acyltriazene (=amide + 28) molecular ions assuming a comparable volatility of both molecular ions (for quantification by NMR see further on in the text). [c] Result from two independent experiments.

deuterium from added D_2O (example **10b** + **10d** + D_2O). All resonances of acyltriazene **14b**, the "exchangeable" as well as the "non-exchangeable" ones (see SI), vanished completely upon prolongued contact with added D_2O (compare pure **14a** with **14a** + **14b** + D_2O in Figure 2).

The ^{19}F resonances of the same amide/acyltriazene mixtures consistently showed a population of several isomeric forms of the latter usually at higher fields than the corresponding amide (Figure 3). The pure azides **9a**, **9b**, **11a**, **11b** and **12** and the pure amides **10a**, **10b**, **2**, **15** and **3** appeared as single isomers showing single peaks in the ^1H-decoupled ^{19}F NMR spectra (Supporting Information), not so the diastereoisomeric and possibly aggregated (hydrogen bonded) forms of **14a** (Figure 3, upper spectrum) or **13** (Supporting Information).[22–32]

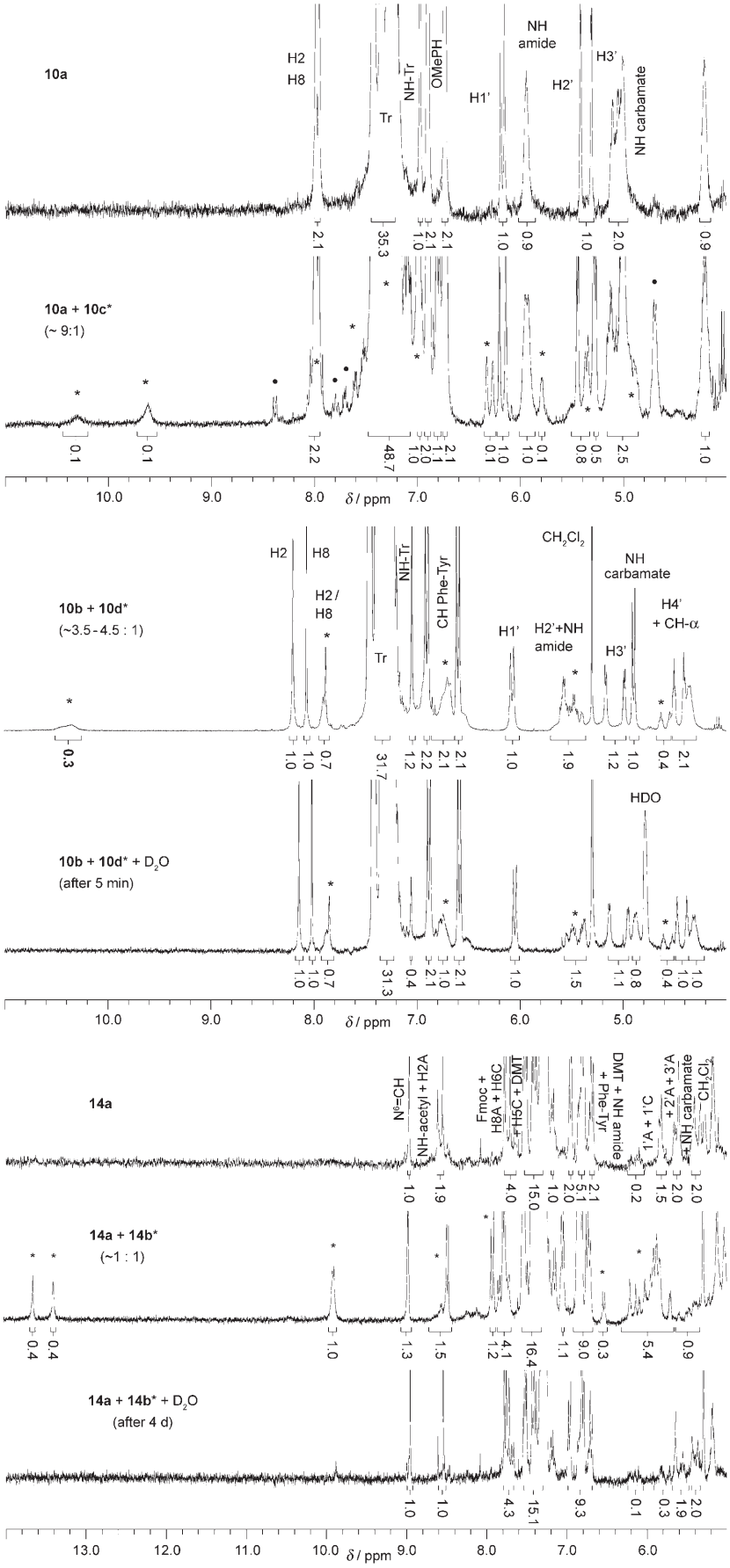
Since the chromatographic separation of the acyltriazenes from the corresponding amides was too difficult on a column or impossible, we resorted to chemical means (Table 2). The mixture **14a** + **14b** was first heated in refluxing 1,4-dioxane which caused the decomposition of all products (entry 1). We replaced dioxane with DMF and found for the mixtures **10a** + **10c** and **14a** + **14b** that at 110°C the amides remained stable whereas the acyltriazenes decomposed (entry 2). After successfully isolating acyltriazene **10d** through preparative TLC, we attempted to convert it to amide **10b** as described by Inazu and collegues[33] and succeeded quite unexpectedly (Table 2, entries 3 and 4).

Discussion

In this synthesis three chemical steps are of particular importance: the stereospecific formation of a nucleosidic *lyxo* 2′,3′-epoxide from a *cis*-vicinal ditriflate, the practical and regioselective ring-opening with inorganic azide and the very high yielding in situ coupling of the organoazide with an active ester of an amino acid. This Staudinger–Vilarrasa coupling, a particularly efficient variant of the so-called modified Staudinger reaction[34] of a which an attractive water-compatible version became known as the Staudinger ligation,[35] is the most important key step, since it bears a great potential in more general synthetic contexts.

The Staudinger reaction[36] is a redox reaction occurring between a phosphine as the reducing agent and an organoazide as the oxidizing agent, to initially produce a phosphatriazene (triazaphosphadiene, phosphazide) by nucleophilic attack of the phosphorus atom of the phosphine at the terminal nitrogen atom (Nα) of the organoazide.[37] In the following step, the intermediate phosphatriazene undergoes an intramolecular rearrangement via a four-membered P-N-N-N-ring transition state to yield a second intermediate, the iminophosphorane, with concomitant loss of N_2. The resulting iminophosphorane is hydrolyzed in the most common version to the amine and phosphorane oxide. The iminophosphorane's highly nucleophilic nitrogen atom, however, can react with almost any kind of electrophilic reagent (the modified versions), thus resulting in many reactions of significant synthetic importance.[34]

Phosphatriazenes: Horner and Gross[38] were the first to investigate the mechanism of the Staudinger reaction. They



showed that in some cases, phosphatriazenes are quite stable under the usual conditions of the Staudinger reaction in organic solvents at 0–2 °C, but evolve dinitrogen upon heating to 50–150 °C. They noticed the formation of phosphatriazene following second-order kinetics. Later Leffler and Temple[39] reinvestigated this result, confirmed it and refined the theory. They postulated a free energy profile of the Staudinger reaction in which the phosphatriazene complex is rather stable, more stable than the reagent pair (PPh_3 + PhN_3), thus, saw the phosphatriazene as a *kinetically trapped, mostly enthalpically stabilized* reaction intermediate. It was also found that the fragment PN_3R in the isolated phosphatriazenes was acyclic, that is, the azide bound to the P^{III} site with Nα, its terminal nitrogen atom. In the isolated compounds the chains PN_3C were nearly planar and had the *trans* (*E*) configuration with respect to the central Nα–Nβ bond which exhibits, partially, double-bond characteristics (references in [37b]). pK_a measurements have shown that phosphatriazenes are less basic than the corresponding iminophosphoranes.[40] As the parameters ρ in the Hammett correlations of pK_a values for phosphatriazenes are much smaller than for the corresponding iminophosphoranes, it was concluded that, for the

Figure 2. Comparison of the downfield region of the ^{1}H NMR spectra in $CDCl_3$ at 298 K of amides and mixtures of amides and acyltriazenes (marked with an asterisk, unidentified resonances marked with a dot). The amide-acyltriazene ratios according to the integration of sufficiently well resolved ^{1}H resonances are: **10a**/**10c** ≈91:9 (H1′ and NH amide); **10b**/**10d** ≈74:26 (H2 + H8), 71.5:28.5 (H4′) and 70.5:29.5 (OCH_3, not shown here, see Supporting Information).

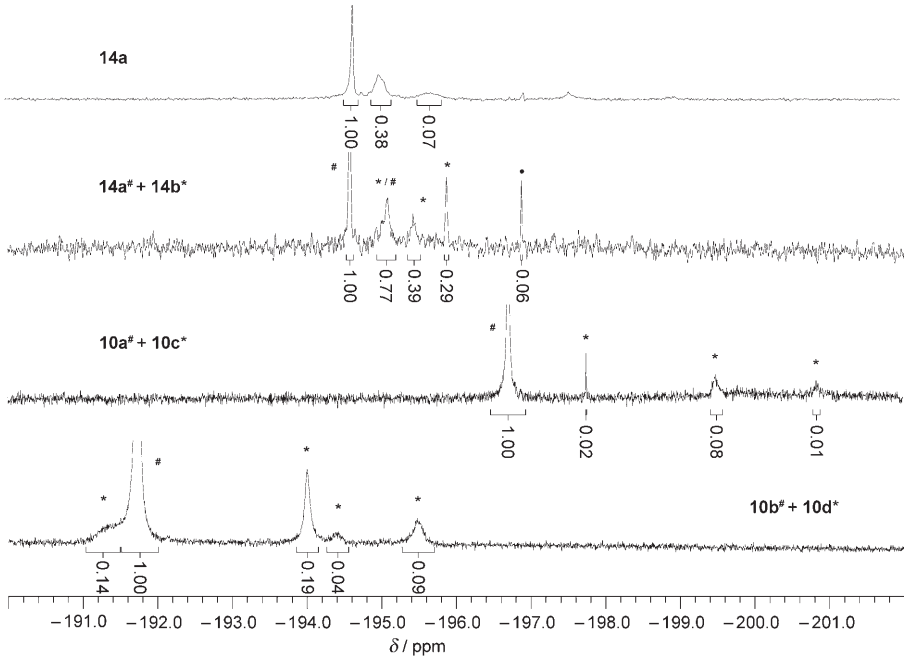


Figure 3. Comparison of the ^{1}H-decoupled ^{19}F NMR spectra in $CDCl_3$ at 298 K of mixtures of amides (#) and acyltriazenes (*). The amide-acyltriazene ratios according to the integration of the ^{19}F resonances are: **14a/14b** ≈59–55:41–45 (F2′); **10a/10c** ≈90:10 (F2′); **10b/10d** ≈69.5:30.5 (F3′). The signal marked with a dot could not be found in the fully coupled ^{19}F NMR spectrum (Supporting Information).

Table 2. Heating of product mixtures (acyltriazene + amide) under various conditions.

Entry	Starting compounds[a]	Reaction conditions	Results[a]
1	53:47 **14a/14b**	dioxane reflux (100 °C), 60 min	**14a** and **14b** decompose
2	91:9 **10a/10c** 53:47 **14a/14b**	DMF 110 °C, 40 min	**10a**, **14a** are stable **10c**, **14b** decompose
3	<5:>95 **10b/10d**	DOWEX-H$^+$ toluene reflux (130 °C), 60 min	58:42 **10b/10d**
4	58:42 **10b/10d**	10 % AcOH toluene reflux (130 °C), 30 min	100:0 **10b/10d**

[a] Identified through TLC and quantified by ESI$^+$ MS of the crude reaction mixtures.

phosphatriazenes, protonation occurs at Nα, the N atom adjacent to phosphorus. Later a few *cis* (*Z*) configured phosphatriazenes have been isolated where steric bulkiness and structural constraints of the carbon backbone seemed to explain the unusual preference to a large part, although a favorable *cis* P···Nγ overlap (weak attractive interaction) may also have contributed.[41]

Phosphatriazenes to iminophosphoranes: The thermal conversion into iminophosphoranes through the mechanism previously studied was experimentally investigated.[36,37a,42] In general the reaction seemed to proceed without either free radicals or nitrene participation[39,43a] and possibly with retention of configuration at the phosphorus atom.[43b] By using ^{15}N labeling techniques Bock and Schnöller[44] demonstrated that the elimination of the two nitrogen atoms from the phosphatriazene were Nα–Nβ and the corresponding product was therefore the iminophosphorane containing Nγ. This demonstration confirmed through experiment that the decomposition of phosphatriazenes is an intramolecular mechanism via a four-membered ring transition state.

In the groups of Molina,[41a] Rzepa,[45] Grützmacher[46] and Tian and Wang[47] the Staudinger reaction profile was investigated by means of ab initio calculations using several methods and two kinds of basis sets: restricted Hartree–Fock (RHF/6-31G*),[41,45] coupled cluster calculations on single and double substitutions from the Hartree–Fock determinant (including non-iteratively calculated triple excitations) using geometries from fully correlated second order Møller–Plesset perturbation calculations (CCSD(T)/6-31G**//MP2(full)/6-31G*)[46] and a popular hybrid Hartree-Fock/density functional theory method (B3LYP/6-31G*[45,47] or B3LYP/6-311G**)[45] that included zero-point vibrational energy corrections and stationary point Gibbs free energies on the reaction profiles and a number of different substituents on phosphorous and Nγ, as well as bulk (continuum) solvent effects.[47]

These studies reconstructed the Staudinger reaction—very briefly—-as one that proceeds through two major steps: i) the system moves fastest through an initial P-Nα-Nβ-NγC *cis*-transition state, $\Delta\Delta G^{\neq}_{298\,K}$ (TS1−reactands) = 21.5–24.9 kcal mol^{-1} for the alkyl-substituted reactands,[47]—improbably slower through a regioisomeric P-Nγ(C)-Nβ-Nα and usually slower still through a P-Nα-Nβ-NγC *trans*-transition state—to form the *cis* intermediate being usually (for most alkyl-substituted reactands) slightly more stable than the *trans* isomer, $\Delta\Delta G_{298\,K}$ (*trans–cis*) = 2.2–4.0 kcal mol^{-1};[47] ii) the *cis* intermediate can either rapidly isomerise to the *trans* intermediate, $\Delta\Delta G^{\neq}_{298\,K}$ (TS1−*cis*) = 10.8–12.7 kcal mol^{-1} for most alkyl-substituted reactands,[47] or it forms a four-membered ring in passing through at least one (or more) transition states, $\Delta\Delta G^{\neq}_{298\,K}$ (TS2−*cis*) = 17.4–26.2 kcal mol^{-1} for most alkyl-substituted reactands,[47] with retention of the original configuration at phosphorus, whereafter N_2 dissociates over a very small or no barrier.

Interestingly, the free energy barriers of *cis–trans* isomerisation and even reversion, $\Delta\Delta G^{\neq}_{298\,K}$ (*cis*TS–*cis*) = 7.5–12.7 kcal mol^{-1} for the alkyl-substituted P-Nα-Nβ-NγC *cis*-phosphatriazenes,[47] are lower than the ones for cyclization

and dissociation of N_2 which makes the formation of this intermediate fully reversible (thermodynamic control). As a result of the flat potential energy surface in the second forward step, that is, the quasi isoenthalpic and isoergonic P···Nγ overlap transition state, P–Nγ ring closure, Nβ···Nγ loosening, followed by the cleavage of Nα≡Nβ (N_2), the Staudinger reaction essentially takes one rate-limiting barrier to form the *cis*-phosphatriazene and another similarly rate-limiting one to rearrange into the iminophosphorane and dissociate N_2. It seems that, unlike Leffler and Temple's presupposition of a kinetically trapped enthalpically favored phosphatriazene (deep energy well, high barriers), this intermediate is relatively labile but may be nevertheless quite abundant in the steady state and thus efficiently *trapped for entropic reasons*. Despite an established P–Nγ contact the system may move back and forth over a number of flat transition states between several *kinetically labile* cyclic intermediate structures along the reaction pathway, it may very easily reopen to the P-Nα-Nβ-NγC phosphatriazene (*cis* or *trans*), even reverse to phosphine and azide, before an appropriate cyclic intermediate eventually dissociates N_2.

To envisage trapping reactions that scavenge the phosphatriazenes into stabilized derivatives that prevent the *cis* intermediate from dissociating N_2, the calculated natural charges on Nα and Nγ in the P-Nα-Nβ-NγC *cis*-phosphatriazenes are of particular interest (Table 3 in ref. [47]). Upon formation of an alkyl-substituted P-Nα-Nβ-NγC *cis*-phosphatriazene it is Nα that faces the strongest rise in electron density—some 0.16 to 0.24 electron charge surplus with respect to the azide, depending on the nature of the substituents. However, the highest absolute negative charge density is always on Nγ, be it in the azide or the P-Nα-Nβ-NγC *cis*-phosphatriazene: −0.31 to −0.61 on Nγ versus −0.14 to −0.29 on Nα of the P-substituted phosphatriazenes (versus −0.04 to −0.07 on Nα of the azides). This questions the formerly predicted preferred protonation site and insinuates comparable nucleophilicities of Nα and Nγ depending on the steric accessibilty of each.

Experimental and theoretical studies on the natural charges in iminophosphoranes showed varied but consistently high charge separations, that is, high negative natural charges on nitrogen and high positive natural charges on phosphorus rationalizing the higher nucleophilicity of iminophosphoranes with respect to phosphatriazenes and suggesting that, in spite of the commonly used double bond ylide-like representation, they are best described as non-hypervalent partly zwitterionic compounds with quite short $N^{-}-P^{+}$ bond lengths owing to electrostatic contraction and possibly n–σ* hyperconjugation.[48]

Acyltriazenes: This class of compounds is well known since 1983, especially since its stabilizing prodrug effect was being explored by Michejda, Smith Jr., Kroeger Smith and collegues,[49] as well as by Rosa and collegues[50] for a number of 1,3-dialkyl- and 1-alkyl-3-aryltriazenes known to be mutagenic due to their DNA alkylation and double-strand cross-linking features. The studied acyl (carbonyl) groups were usually simple, derived from acetate or succinate or carbonic acid derivatives (carbamates and ureas), the role of which was to slow down the too rapid formation of the DNA damaging alkyldiazonium salts and concomitantly amines (for instance, DNA damaging β-chloroethylamine) in unacylated triazenes; the diacyls also served as linkers between triazene and signal peptides. Many of these studies were devoted to the hydrolytic decomposition—most prominently, enzymatic or basic acyl hydrolysis versus acidic cleavage of the RNN···NC(O)R′ bond to give the amide and the diazonium salt—metabolisation, nucleobase and DNA damaging mechanisms of acyltriazenes which were compared with the ones of the related triazenes.[51] The aqueous acid-induced fragmentation of 3-acyl-1,3-dialkyl[49d] and 3-acyl-3-alkyl-1-aryltriazenes[50a] into the respective diazonium salts and amides was experimentally observed. RHF/3-21G calculations in vacuo proposed conformational preferences, confirmed a generally reduced basicity with respect to the unacylated triazenes and suggested the major proton affinities to be at N1 (Nγ) and the carbonyl oxygen atom, not N3 (Nα) directly linked to the carbonyl group.[49e] Consequently, the formation of RN_2^{+} and R′NHC(O)R″ from RNγ=Nβ-Nα(R′)C(O)R″ was proposed to be preceded by the protonation of the carbonyl oxygen atom,[49d] but the fragmentation behavior of certain 3-acyl-3-alkyl-1-aryltriazenes questioned the generality of this assumption.[50a]

Some α-aminoacyltriazenes were studied as perhaps better prodrugs with respect to their solubility in water.[50d] Another strategy was followed by Vaughan and collegues, who investigated 1-aryl-3,3-dialkyltriazenes, bistriazenes, triazines and triazinones.[52] Triazinones can be considered as cyclic acyl- or carbonyltriazenes. Interestingly, they found their way into the clinics as antitumor drugs against, for instance, brain tumors and metastatic melanoma.[53] None of the above pharmacologically highly interesting and useful compounds were synthesized from organoazides using the Staudinger route.

Inazu and collegues reported, in their attempts to synthesize *N*-β-glucopyranosyl *N*γ-asparagines and *N*δ-glutamines, that is, carboxamides from glucopyranosyl azides and unactivated carboxylic acids derived from aspartate[54] and glutamate, thus, following the original Staudinger–Vilarrasa conditions,[34a] on the formation of an acyltriazene (a protected derivative of *N*γ-glucopyranosylazo-L-asparagine) in varying amounts at low temperatures and using simple trialkylphosphines, not phosphites.[33] The authors did not specify the configuration of the acyltriazene's N=N double bond but described the conversion of the acyltriazene to the *N*-glucopyranosyl amide using non-aqueous acid and heat (AcOH or acidic cation exchange resin in refluxing toluene) without proposing a mechanism that would explain this intramolecular way of eliminating N_2 and concomitant 1,3-shift of the acyl group. This reaction clearly contrasts the aqueous acid-induced fragmentation of acyltriazenes into diazonium salts and not rearranged amides or deacylation followed by elimination of N_2 from the resulting triazenes to give amines and alcohols, as described earlier.

All *N*-acyl-*N*-alkyltriazenes described in the literature are assumed to bear a linear acyl-NNN-C constitution. The only structural proof of it stems from a crystal structure of a benzoyltriazene that was obtained from the benzoylation of a quite stable monoalkyltriazene that was isolated from the incomplete catalytic hydrogenation in ethyl acetate of the corresponding azide into the desired amine by Gaoni (Figure 4),[55] thus, following the classical Staudinger route.[36] The isolated monalkyltriazenes were exceptionally stable.

Cmoch identified in a [D_6]DMSO solution of selectively labeled [1-^{15}N]-1-carbamoyl-3-(tetrazolo[1,5-*b*]pyridazin-6-yl)triazene one single isomer, the N1*H* tautomer of unidentified stereochemistry around N2=N3, thus, a strong preference for the H_2N-CO-NH-N=N-R over the H_2N-CO-N=N-NH-R tautomer, R being an aromatic *N*-heterocycle. The compound was obtained from acid hydrolysis of the corresponding 1-cyanotriazene using 6 N HCl in acetone at 60 °C, proved therefore exceptionally stable as well.[56]

Triazenes: Tautomers and *cis–trans* (*E–Z*) isomers of unacylated triazenes were studied experimentally[31b,51b,57] and theoretically.[31b,58] In particular, Khramov and Bielawski obtained the X-ray crystal structures of both *Z* and *E* isomers of the same compound, 1-benzyl-3-(1,3-dimesitylimidiazol-2-ylidene)triazene.[57b] A crystal that contained the *Z* isomer was obtained only once. In the crystal structures of both *Z* and *E* the molecules occupied two crystallographically unique positions in the asymmetric unit. In the crystal containing the *Z* isomer this isomer was ordered in one crystallographically unique position while in the other position it was found partly disordered around the triazene moiety. In the *E* crystal both crystallographically unique positions were ordered and *E* configured. In solution, the isomerisation between the *E* and *Z* forms could not be resolved by ^{1}H NMR at −80 °C in [D_8]toluene. Most interestingly, the authors managed to extrude N_2 from the compound to obtain the *N*-benzyl-1,3-dimesityl-2-iminoimidazoline in >95 % yield through heating of the triazene in DMSO above 120 °C. To the best of our knowledge, this is the only other example of an intramolecular N_2 elimination and concomitant 1,3-alkyl- or -acyl shift involving one carbon and three nitrogen atoms, apart from the one reported by Inazu and collegues[33] and our preliminary results reported here.[59–61]

Although Khramov and Bielawski's crystalline triazene differs somewhat from the ones discussed above, since it is a 1-alkyl-3-aryl*idene*triazene rather than a 1-alkyl-3-aryl- or a 1,3-dialkyltriazene, or 1-acyl-3-aryl- or -alkyltriazene for that matter, hence contains two formal double bonds (R=N−N=N−R′) rather than one, it strongly suggests through experiment that, most probably, triazenes and perhaps also acyltriazenes, unlike possibly many phosphatriazenes in the non-solid state,[47] prefer the *E* (*trans*) configuration around the (partial) N=N double bond, although the *Z* (*cis*) configuration may be in some cases almost equally well accessible and, more importantly, that the *E–Z* isomerization is always a fast process at ambient temperature.

This was experimentally confirmed by Limbach and collegues[31b] who showed in a series of impressingly minute and precise NMR experiments an estimated preference of the *E* over the *Z* isomer of 1,3-di-(*p*-fluorophenyl)triazene of some 300:1 through the integration of ^{19}F resonances that could be resolved at −35 °C but coalesced at 25 °C. Their

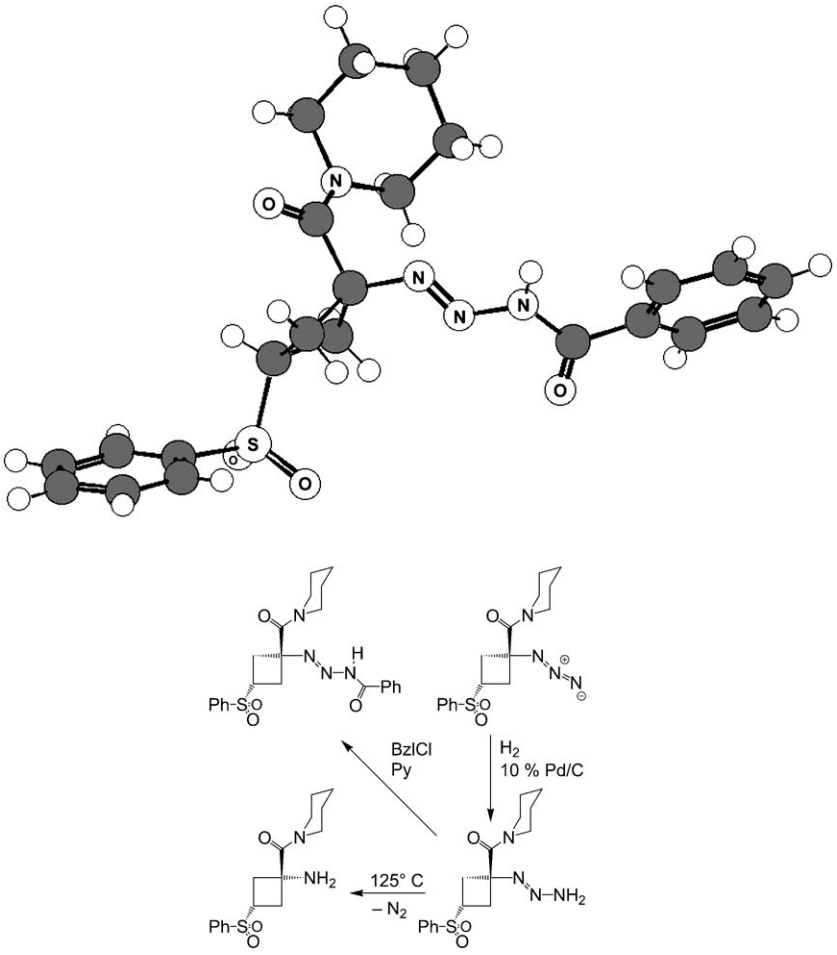


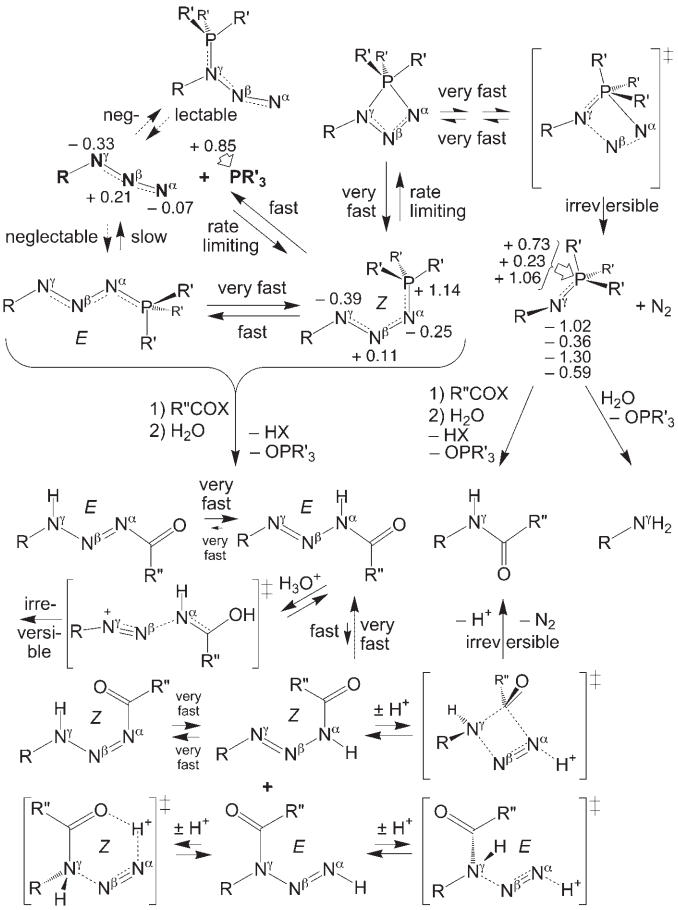
Figure 4. Top: Crystal structure TUGGOR (from CCDC) showing the linear s-*trans* arrangement of the benzoyltriazene. Bottom: Reaction scheme taken from ref. [55].

main objective was to study, using small amounts of dimethyl- and trimethylamine, the base-catalyzed tautomerisation mechanism of the N*H* tautomers of FPh-NH-N=N-PhF, the ^{1}H NMR resonance of which appeared at δ 11.4 or around 12 ppm (compare to Figure 2). It could only be "frozen out of exchange" (out of line broadening and coalescence) below some −125 °C. The authors elucidated an experimental 5.5 kcal mol^{-1} tautomerisation free energy barrier and concluded from preliminary hybrid HF-DFT calculations (B3LYP/6-31+G**), taking into account the polarized continuum solvent method and the self-consistent reaction field for chloroform and tetrahydrofuran, that the fastest passage for the proton happened with the help of a base-assisted hydrogen-bond switching through a transition state without dissociation of the triazene-base contact ion pair. The experimental barrier is in agreement with theoretical studies on water-assisted 1,3-hydrogen shifts in triazene proper,[58b] while earlier ^{1}H NMR measurements suggested tautomerisation free energy barriers at 295 K of various 1,3-dialkyltriazenes in methanol of, according to the transition state theory, 12.8–16.0 kcal mol^{-1}.[51b]

Trapping phosphatriazenes before and without the loss of N_2: Taken together all above studies, we can describe the various possible reactivities in Scheme 5. The upper half of the scheme, that is, the reactions before the production of phosphorane oxide, is much more certain than the reactions in the lower half of the scheme. However, it is the lower half that describes the access to many potentially valuable products. In the context of the synthesis presented here we have to explain two observed results: A) Why do we see in the NMR spectra of the solutions that contain acyltriazenes, at ambient temperature, more than one proton resonance at low fields and more than one fluorine resonance at higher fields than for those of the amides (Figures 2 and 3)?

If all studies on triazenes and acyltriazenes point to very rapid *E*–*Z* isomerizations and N1−N3 prototropies, we are forced to conclude that we may have obtained constitutional isomers that could not be in thermodynamic equilibrium. Alternatively, we did observe several *E*–*Z* isomers and/or N1−N3 tautomers indeed but their interconversion was significantly slowed down by aggregation through stable hydrogen bonding. Our earlier experience with hydrogen-bonded systems in chloroform[62] contradicts this latter possibility but we cannot exclude it completely. B) Trapping an *E* or *Z* configured phosphatriazene with an acyl group is expected to result in a thermodynamically controlled mixture of rapidly interconverting *E* and *Z* *N*α-acyltriazenes where the Nα*H* tautomers should prevail. Nevertheless, how can we be certain that only Nα of a P-Nα-Nβ-NγC phosphatriazene will react with the electrophile? Thus far the formation of the regioisomeric *N*γ-acyltriazene (lowest central compound in Scheme 5) from the *N*α-phosphatriazene has not been described despite the theoretically obvious nucleophilicity of Nγ.

The best studied degradation pathway of acyltriazenes under neutral or slightly acidic conditions is the fission of



Scheme 5. The Staudinger reaction and variants. *E* and *Z* refer to the configuration between Nβ and Nα (N2 and N1) bearing significant double-bond character. The semiquantitative rate descriptions (related to the calculated free energies at 298 K and 1 atm) and structures before trapping with water or R″COX (X = leaving group) were taken and summarized from Tian and Wang,[47] so were the natural charges on P and N atoms of the reactants and the *cis*-phosphatriazene that refer to the calculated system $Me_3P + N_3Me \Leftrightarrow Me_3PN_3Me$. The Mulliken charges on the iminophosphorane were taken from (first line) H_3PNH,[48b] (second line) Me_3PNH,[48a] (third line) H_3PNH,[48d] and (fourth line for N) Me_3PNH.[48f] Not specified partial charges were not replaced by formal ones. The semiquantitative rate descriptions after trapping refer to NMR experiments with carbamoyl and unacylated triazenes, the corresponding structures are related to the ensemble of previous studies (see text). The structures of the transitions states ≠ after trapping with R″COX and before fragmentation are mere suggestions. The positive charges in the transition states were placed in anticipation of the products formed (diazonium salt and *N*α-amide not shown).

the Nα···Nβ bond catalyzed by hydronium ions and leading to *N*α-carboxamides and highly electrophilic and mutagenic *N*β-*N*γ-diazonium compounds that either alkylate or hydrolyze or eliminate to more stable follow-up products. We seem to have observed this reaction after adding D_2O to a chloroform solution containing amide **14a** and triazene **14b**. After a few days, only **14a** remained (lowest spectrum in Figure 2). Like the formerly studied 3-acyl-1,3-dialkyl[49d] and 3-acyl-3-alkyl-1-aryltriazenes,[50a] **14b** was not stable in contact with water over a long period of time. The proposed protonation of the oxygen atom of acyltriazenes[49d] in aque-

ous solutions seems indeed to be a reasonable assumption and is depicted in Scheme 5.

The formation of the rearranged *N*γ-carboxamides under acidic anhydrous conditions, as observed here and by Inazu and collegues,[33] is more difficult to understand. It necessitates the protonation of Nγ bearing a relatively high calculated proton affinity,[49e] to loosen the carbonyl···Nα, as well as the Nβ···Nγ bonds and to attract the carbonyl group towards Nγ. Such a rearrangement needs to pass through a four-membered ring transition state, as depicted in Scheme 5, a reaction pathway similar, in a sense, to the rearrangement of *N*-alkyl-*N*-nitrosoamides into *N*-alkyl-diazoesters that subsequently eliminate dinitrogen to form *O*-alkyl esters.[60,61] If, however, no rearrangement is needed to form the experimentally observed *N*γ-carboxamides because the phosphatriazene reacted directly to the regioisomeric *N*γ-acyltriazene in the first place, then at least two reasonable Nγ-protonated transition states would lead to the elimination of dinitrogen, as shown in the lowest part of Scheme 5.

In view of the fact that we observed in the NMR spectra of our acyltriazenes at ambient temperature several fluorine resonances (Figure 3) and several distinct water-exchangeable low-field proton resonances, two of which between δ 13 and 14 ppm (Figure 2) where usually only hydrogen-bonded systems resonate (such as adenosine-paired uridine imino protons), we are inclined to take those regioisomeric *N*γ-acyltriazenes into consideration, at least in a mixture containing *N*α- and *N*γ-acyltriazenes. Whereas R-Nγ=Nβ-NαH-COR′ possibly "looks more stable", R-Nγ(COR′)-Nβ=Nα-H seems to be ideally suited to form various hydrogen-bonded aggregates in chloroform, with themselves, with *N*α-acyltriazenes, as well as with *N*γ-carboxamides, perhaps just stably enough to be observed as distinct objects on the NMR time scale.

In the COSY of the 78:22 **10b + 10d** mixture (Table 1, entry 3) we found a clear correlation (Supporting Information, page 67) between a H2′ resonance at δ 5.5 ppm (showing a large vicinal H–F coupling) and a resonance at 6.6 ppm (both marked with asterisks in Figure 2) that appeared smaller in the acyltriazene-depleted 9:1 **10b + 10d** mixture (Supporting Information, page 72) and was water-exchangeable, hence, rapidly vanished upon contact with D_2O (Supporting Information, page 70). We therefore assigned the water-exchangeable part of the 6.6 ppm resonance to an amide hydrogen atom of the major acyltriazene. Whereas in 1-carbamoyl-3-aryltriazenes there seems to be a strong preference for the H_2NCO-NH-N=N-Ar over the H_2NCO-N=N-NH-Ar tautomer,[56] we might have been observing, rather than R′CO-NH-N=N-R, tautomeric R′CO-N=N-NH-R where R contains H2′ vicinal to R′CONNN*H*. Of course, only a combination of NMR experiments on specifically ^{15}N-labeled acyltriazenes and high-level ab initio calculations will be able to fully answer these open questions.

Conclusion

Two new fluorinated puromycin analogues were synthesized with the purpose of testing their protein synthesis inhibiting activity in vitro and antibiotic activity in procaryotes. An optimized seven and thirteen steps procedure is presented for, respectively, **2** and **3** from adenosine which includes a very efficient Staudinger–Vilarrasa coupling reaction between an organoazide and the 1-oxybenzotriazole ester of an amino acid derivative. The coupling reaction leads to carboxamides in 94% isolated yield under very mild reaction conditions at ambient temperature and is therefore likely to be of more general value than presented here, for instance, as an alternative for obtaining sterically demanding peptides from azides not amines.[34t]

The same reaction at lower temperatures generates mixtures of carboxamides and acyltriazenes of a priori unknown tauto-, stereo- and regioisomery. A careful analysis of the literature on the Staudinger reaction and acyltriazenes revealed that this medicinally highly valuable class of compounds—small water-soluble cyclic carbonyl triazene compounds are among the rare clinical cancerostatics that are able to pass the blood–brain barrier and that also cure metastatic melanoma—never has been approached by through the Staudinger reaction. The assumed mechanism of action of acyltriazenes at physiological or slightly acidic pH is the proton-assisted cleavage of a nitrogen−nitrogen bond such that a carboxamide and an alkyl or aryl diazonium salt are generated in situ alkylating nucleic acids of which the ultimately crosslinked DNA double strands become lethal to the affected cell.

We tested here different, anhydrous proton-assisted acyltriazene cleavage conditions and obtained, after the elimination of dinitrogen, different carboxamides that formally but not necessarily resulted from a N1,N3 shift of the aminoacyl group. Since the chemical reaction mechanism is hardly known, we publish, interpret and discuss all spectroscopic data on our compounds and compare them to what was known before, in order to gain a maximum insight into a synthetically attractive variant of the Staudinger reaction and into an exciting class of compounds, the acyltriazenes. Last but not least, we wish to enrich the literature, using all our fluorinated compounds, with fully interpreted[22] ^{19}F NMR spectra (besides ^{1}H, ^{13}C and ^{31}P), a research tool still in its youth despite the rapidly growing number of medicinally valuable fluorinated organic compounds. The target compounds will be subjected to biological assays for antibiotic activity.

Experimental Section

General: Pyridine was dried over CaH_2 and freshly distilled, CH_2Cl_2 and THF were dried over molecular sieves 4 Å. Other reagents were used as purchased. ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded in $CDCl_3$, [D_6]DMSO, CD_3OD, and H_2O/D_2O (5%), at 300.1, 75.5, 282.4, and 121.5 MHz, respectively. Chemical shifts are given in ppm relative to residual $CHCl_3$ (δ7.26) or CH_2Cl_2 (δ5.29) or CHD_2OD (δ3.31) or CH_3OH

(δ3.44) or CHD_3SOCD_3 (δ2.50) for ^1H, $CDCl_3$ (δ77.23) or CD_3OD (δ49.00) for ^{13}C as internal references, and $CFCl_3$ (δ0) for ^{19}F and H_3PO_4 (δ0) for ^{31}P as external references. Signals were attributed based on H–D exchange (^1H), COSY, DEPT (^{13}C) and HSQC spectra. Signal shapes and multiplicities: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, sext = sextuplet, m = multiplet. Scalar coupling constants J are given in Hertz (Hz). Mass spectra (MS and HRMS) were obtained using electron ionisation (EI) and chemical ionisation (CI), fast atomic bombardment (FAB from CH_2Cl_2 or H_2O/MeOH 9:1) and electrospray ionization (ESI, from CH_2Cl_2 or H_2O/MeOH 9:1), in part with time-of-flight detection (TOF). Infrared spectra were obtained on a Bruker IFS 66, Perkin-Elmer 681. Flash chromatography was performed on silica gel 60 (0.04–0.063 mm). Thin-layer chromatography (TLC plate, Merck, silica gel on Aluminium, 20X) was performed on a pre-coated silica gel F254 plates with fluorescent indicator. The detection of compounds was carried out with UV light (254 nm). Nucleosides were visualized on TLC plates by subsequent spraying with concentrated H_2SO_4 and 2% naphtoresorcinol solutions in ethanol, followed by heating. UV/Vis spectra were recorded on a Perkin-Elmer Lambda Bio 40 spectrophotometer equipped with a deuterium and tungsten-halogen lamp. The buffer solutions were prepared with water purified through the Nanopure Ultrapure D4742 water system of Barnstead. The salts (biochemical quality) were obtained from Fluka. The melting points (m.p.) and, for amorphous solids, melting ranges (m.r.) were determined in capillary tubes heated electrically in a silicon oil bath (Büchi apparatus), are given in degree Centigrade and are uncorrected. Analytical RP-HPLC purification: 250×8 mm Eurospher 100/5 RP_{18} column (Knauer), flow rate: 1.3 mL min^{-1}; UV detection at 260 nm. Eluants for HPLC were prepared with water purified through the Milli-Q system. CH_3CN was HPLC grade: A) H_2O, TFA 0.05 M, CH_3CN 1%; B) CH_3CN/H_2O 9:1.

6-*N*,5′-*O*-Ditrityladenosine (5): A mixture of adenosine (**4**) (10 g, 37.42 mmol) dried by coevaporation with pyridine, DMAP (3.84 g, 31.43 mmol), and TrCl (35 g, 125.35 mmol) in pyridine (500 mL) was heated at 80 °C. The progress of the reaction was followed by TLC (EtOAc/MeOH 9:1). After 4 h 40 min the reaction was cooled down to ambient temperature and quenched with EtOH (150 mL). The reaction mixture was concentrated in vacuo and coevaporated with toluene (2× 250 mL). The residue was suspended in toluene (250 mL), well shaken, filtered and the precipitate rinsed several times with toluene. The filtrate was concentrated in vacuo, the residue was dissolved in a minimal amount of CH_2Cl_2, a mixture of toluene and EtOAc (85:15, 75 mL) was added, then concentrated under reduced pressure until opaqueness occurred, and the solution was kept at room temperature to recrystallize **5**. Compound **5** (7.2 g) was filtered after 3.5 h and the filtrate was applied on a column of silica gel (MePh/EtOH 4:1:0.2) to give addditional amounts of **5** (2.2 g). Total yield: 34%; white solid. M.p. 213–216 °C (214–217 °C);[15b] R_f = 0.37 (MePh/EtOAc/EtOH 4:1:0.2); ^1H NMR ([D$_6$]DMSO): δ = 8.36 (s, 1H, H-2), 7.82 (s, 1H, H-8), 7.46 (s, 1H, N^6-H), 7.36–7.18 (m, 30H, 6×C_6H_5), 5.91 (d, 1H, ^3J(1′,2′) = 4.7 Hz, H-1′), 5.52 (d, 1H, ^3J = 5.7 Hz, OH-3′/2′), 5.21 (d, 1H, ^3J = 5.7 Hz, OH-2′/3′), 4.73 (q, 1H, ^3J = 5.2 Hz, H-2′), 4.29 (q, 1H, ^3J = 5.3 Hz, H-3′), 4.06 (q, 1H, ^3J = 4.7 Hz, H-4′), 3.20 ppm (br d, 2H, J = 4.5 Hz, H-5″ + H-5′); ^{13}C NMR ($CDCl_3$): δ = 154.4, 151.7, 148.1, 145.0, 143.5, 138.3, 129.2, 128.7, 128.1, 128.1, 127.4, 127.2, 121.4, 91.2, 87.3, 86.6, 76.3, 73.2, 71.7, 63.9 ppm; HRMS (FAB$^+$): m/z: calcd for $C_{48}H_{41}N_5O_4$: 751.3159; found: 751.3160 $[M]^+$.

2′,3′-Di-*O*-trifluoromethylsulfonyl-6-*N*,5′-*O*-ditrityladenosine (6a): Compound **5** (2.30 g, 3.06 mmol) and DMAP (0.935 g, 7.65 mmol) were dissolved in pyridine (70 mL). This solution was cooled to 0 °C and a solution of triflic anhydride (4 mL, 24.48 mmol) was added in a dropwise manner. The reaction mixture was held at 0 °C for 30 min and then allowed to warm to room temperature over a period of 3 h. The reaction mixture was then added to CH_2Cl_2 (200 mL) extracted with water (200 mL at 0 °C). The solvents were removed under reduced pressure and the residue purified by chromatography on a column of silica gel (MePh/EtOAc 9:1) to give **6d** (0.40 g, 15%) and **6a** (2.60 g, 83.7%) as a white amorphous solid. **6a**: M.r. 92–102 °C; R_f = 0.58 (MePh/EtOAc 9:1); ^1H NMR ($CDCl_3$): δ = 7.88 (s, 1H, H-2), 7.83 (s, 1H, H-8), 7.38–7.16 (m, 30H, 6×C_6H_5), 6.98 (s, 1H, N^6-H), 6.45 (t, 1H, $^3J \approx$ 5.4 Hz, H-2′), 6.21 (d, 1H, ^3J(1′,2′) = 5.9 Hz, H-1′), 5.83 (dd, 1H, ^3J = 5.2, $^3J \approx$ 2.4 Hz, H-3′), 4.48 (dd, 1H, ^3J = 3.9, ^3J = 7.4 Hz, H-4′), 3.68 (dd, 1H, ^3J = 4.2, ^2J = 11.2 Hz, H-5′), 3.36 ppm (dd, 1H, ^3J = 3.9, ^2J = 11.2 Hz, H-5″); ^{19}F NMR ($CDCl_3$): δ = −74.55, −74.69 (2s, 2×CF_3); ^{13}C NMR ($CDCl_3$): δ = 154.6, 152.9, 148.7, 145.0, 143.1, 139.0, 129.2, 128.7, 128.2, 128.1, 127.7, 127.2, 121.7, 118.5 (q, ^1J = 320.0 Hz), 118.3 (q, ^1J = 320.0 Hz), 88.1, 85.4, 81.8, 81.7, 80.4, 71.8, 61.7 ppm; HRMS (FAB$^+$): m/z: calcd for $C_{50}H_{39}F_6N_5O_8S_2$: 1015.2144; found: 1015.2142 $[M]^+$.

9-(2′,3′-Anhydro-6-*N*,5′-*O*-ditrityl-β-D-lyxofuranosyl)adenine (7): Compound **6a** (2.6 g, 2.56 mmol) was dissolved in toluene (55 mL) containing $Bu_4N^+NO_2^-$ (5.9 g, 20.48 mmol) and water (7 mL). After vigorously stirring the reaction mixture for 40 h, the reaction mixture was extracted with tBuOCH$_3$ (90 mL) and water (2×130 mL), dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo under reduced pressure. The residue was purified by chromatography on a column of silica gel (MePh/EtOAc 9:1 → MePh/EtOAc 8:2) to give **6c** (227 mg, 10%) and **7** (1.190 g, 64%) as a white amorphous solid. **7**: M.r. 105–115 °C; R_f = 0.51 (MePh/EtOAc 8:2); ^1H NMR ($CDCl_3$): δ = 8.04, 8.02 (2s, 2H, H-2, H-8), 7.46–7.15 (m, 30H, 6×C_6H_5), 6.95 (s, 1H, N^6-H), 6.28 (s, 1H, H-1′), 4.22 (t, 1H, ^3J(4′,5′/5″) = 6.3 Hz, H-4′), 4.02, 4.01 (2d, 2H, ^3J = 5.8 Hz, H-2′, H-3′), 3.49 (dd, 1H, ^3J = 6.2, ^2J = 9.3 Hz, H-5′), 3.37 ppm (dd, 1H, ^3J = 6.5, ^2J = 9.4 Hz, H-5″); ^{13}C NMR ($CDCl_3$): δ = 154.3, 152.6, 148.9, 145.1, 143.8, 139.1, 129.2, 128.8, 128.1, 128.1, 127.4, 127.1, 120.5, 87.3, 81.0, 81.0, 71.6, 62.4, 57.4, 56.7 ppm; HRMS (FAB$^+$): m/z: calcd for $C_{48}H_{39}N_5O_3$: 733.3053; found: 733.3048 $[M]^+$.

9-(3′-Azido-3′-deoxy-6-*N*,5′-*O*-dityrityl-β-D-arabinofuranosyl)adenine (8a) and 9-(2′-azido-2′-deoxy-6-*N*,5′-*O*-dityrityl-β-D-arabinofuranosyl)adenine (8b): A mixture of **7** (925 mg, 1.26 mmol), NH_4Cl (135 mg, 2.52 mmol), NaN_3 (492 mg, 7.56 mmol), DMF (4 mL), and H_2O (600 μL) was heated under reflux at 100 °C for 1 h 30 min. The reaction mixture was extracted with CH_2Cl_2 (35 mL) and water (35 mL). The organic layer was washed with water (3×35 mL), dried with anhydrous $MgSO_4$ and concentrated in vacuo under reduced pressure. The residue was purified by chromatography on a column of silica gel (MePh/EtOAc 8:2 → MePh/EtOAc 7:3) to give **8a** (770 mg, 78.6%) and **8b** (174 mg, 17.8%) as white amorphous solids.

Compound **8a**: M.r. 114–118 °C; R_f = 0.38 (MePh/EtOAc 8:2); ^1H NMR ($CDCl_3$): δ = 8.00, 7.98 (2s, 2H, H-2, H-8), 7.35–7.15 (m, 30H, 6×C_6H_5), 7.05 (s, 1H, N^6-H), 6.04 (d, 1H, ^3J(1′,2′) = 5.3 Hz, H-1′), 5.5 (br d, 1H, ^3J = 9.4 Hz, OH-2′), 4.58–4.51 (br, 1H, H-2′), 4.43 (t, 1H, ^3J(3′,4′) = ^3J(3′,2′) = 6.3 Hz, H-3′), 3.87 (td, 1H, ^3J(4′,5′/5″) = 3.9, ^3J(4′,3′) = 6.4 Hz, H-4′), 3.43 (dd, 1H, ^3J = 3.5, ^2J = 10.7 Hz, H-5′), 3.27 ppm (dd, 1H, ^3J = 4.2, ^2J = 10.7 Hz, H-5″); ^{13}C NMR ($CDCl_3$): δ = 154.7, 152.1, 148.0, 144.9, 143.5, 140.8, 129.2, 128.8, 128.1, 127.5, 127.2, 121.4, 87.5, 85.4, 80.1, 77.1, 71.8, 65.8, 63.1 ppm; IR (CH_2Cl_2): ν = 2100 cm^{-1} (N_3 st); HRMS (FAB$^+$): m/z: calcd for $C_{48}H_{40}N_8O_3$: 776.3223; found: 776.3225 $[M]^+$.

Compound **8b**: M.r. 119–122 °C; R_f = 0.35 (MePh/EtOAc 9:1); ^1H NMR ($CDCl_3$): δ = 7.88, 7.83 (2s, 2H, H-2, H-8), 7.48, 7.44 (d, 1H, ^2J(OH,3′) = 11.1 Hz, OH-3′), 7.35–7.16 (m, 30H, 6×C_6H_5), 7.09 (s, 1H, N^6-H), 5.60 (d, 1H, ^3J(1′,2′) = 2.1 Hz, H-1′), 4.48 (d, 1H, ^3J = 2.1 Hz, H-2′), 4.24, 4.20 (dd, 1H, ^2J(3′,OH) = 11.1, ^3J(3′,4′) = 3.2 Hz, H-3′), 4.18–4.13 (m, 1H, H-4′), 3.54 ppm (d, 1H, ^3J = 5.6 Hz, H5′ + H5″); ^{13}C NMR ($CDCl_3$): δ = 154.6, 151.6, 146.7, 144.7, 143.9, 139.9, 129.1, 128.8, 128.1, 127.9, 127.2, 127.1, 122.0, 90.1, 87.3, 82.7, 75.7, 72.4, 71.7, 62.1 ppm; IR (CH_2Cl_2): $\tilde{\nu}$ = 2114.2 cm^{-1} (N_3 st); HRMS (FAB$^+$): m/z: calcd for $C_{48}H_{40}N_8O_3$: 776.3223; found: 776.3219 $[M]^+$.

3′-Azido-2′,3′-dideoxy-2′-fluoro-6-*N*,5′-*O*-dityrityl-adenosine (9a): Diethylamino sulfur trifluoride (DAST, 0.9 mL, 6.87 mmol) was added in a dropwise manner to a solution of **8a** (835 mg, 1.075 mmol) in toluene (14 mL) and pyridine (1.5 mL), and stirred at room temperature for 30 min before heating the reaction mixture to 80 °C. After 45 min EtOAc (70 mL) was added and the organic layer washed successively with 7% $NaHCO_3$ (60 mL) and water (60 mL), dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo under reduced pressure. The residue was purified on a silica gel column (MePh/EtOAc 8:2) to give recovered **8a** (44 mg, 5.3%) and **9a** (626 mg, 75%) as a yellowish amorphous solid. **9a**: M.r. 106–112 °C; R_f = 0.76 (MePh/EtOAc 8:2); ^1H NMR ($CDCl_3$): δ = 8.07, 7.97 (2s, 2H, H-2, H-8), 7.46–7.26 (m, 30H, 6×C_6H_5), 7.09 (s, 1H, N^6-H),

6.18 (dd, 1H, $^3J(1',2')=1.3$, $^3J(1',F)=19.9$ Hz, H-1′), 5.92 (ddd, 1H, $^2J(2',1')=1.2$, $^3J(2',3')=4.6$, $^3J(2',F)=52.9$ Hz, H-2′), 4.84 (ddd, 1H, $^3J(3',2')=4.7$, $^3J(3',4')=8.7$, $^3J(3',F)=22.7$ Hz, H-3′), 4.34 (td, 1H, $^3J(4',5'/5'')=3.5$, $^3J(4',3')=8.7$ Hz, H-4′), 3.66 (dd, 1H, $^3J(5',4')=3.1$, $^2J(5',5'')=11.0$ Hz, H-5′), 3.42 ppm (dd, 1H, $^3J(5'',4')=4.0$, $^2J(5'',5')=11.0$ Hz, H-5″); ^{19}F NMR ($CDCl_3$): $\delta=-197.79$ ppm (ddd, $^3J(F,3')=22.5$, $^3J(F,1')=19.8$, $^2J(F,2')=52.8$ Hz, F-2′); ^{13}C NMR ($CDCl_3$): $\delta=154.4$, 152.7, 148.3, 145.0, 143.5, 139.3, 129.1, 128.7, 128.1, 128.1, 127.4, 127.1, 121.6, 94.1 (d, $^1J(F,C2')=189.7$ Hz, C2′), 88.2 (d, $^2J(F,C1')=34.3$ Hz, C1′), 87.2, 80.4 (s, $^3J(F,C4')<1$ Hz, C4′), 71.6, 62.1, 59.6 ppm (d, $^2J(F,C3')=15.6$ Hz, C3′); IR (CH_2Cl_2): $\tilde{\nu}=2112.7$ cm^{-1} (N_3 st); HRMS (ESI$^+$ TOF): m/z: calcd for $C_{48}H_{40}F_1N_8O_2$: 779.3258; found: 779.3272 $[M+H]^+$.

2′-Azido-2′,3′-dideoxy-3′-fluoro-6-*N*,5′-*O*-dityladenosine (9b): The same procedure as for **8a** was applied to **8b** (35 mg, 0.045 mmol) to give after colmun chromatography **9b** (29 mg, 83%) as a yellowish amorphous solid. M.r. 108–114°C; $R_f=0.51$ (MePh/EtOAc 9:1); ^{1}H NMR ($CDCl_3$): $\delta=7.88$, 7.84 (2s, 2H, H-2, H-8), 7.40–7.21 (m, 30H, $6\times C_6H_5$), 6.95 (s, 1H, N^6-H), 6.00 (d, 1H, $^3J(1',2')=7.9$ Hz, H-1′), 5.26 (brddd, 1H, $^3J(3',2')=4.5$, $^3J(3',4')<1.3$, $^2J(3',F)=53.5$ Hz, H-3′), 5.18 (ddd, 1H, $^3J(2',1')=7.8$, $^3J(2',3')=4.5$, $^3J(2',F)=23.5$ Hz, H-2′), 4.45 (td, 1H, $^3J(4',F)=25.6$, $^3J(4',5'/5'')=4.3$ Hz, H-4′), 3.51 (dd, 1H, $^3J=5.1$, $^2J=10.7$ Hz, H-5′), 3.39 ppm (dd, 1H, $^3J=4.3$ Hz, $^2J=10.7$ Hz, H-5″); ^{19}F NMR ($CDCl_3$): $\delta=-195.15$ ppm (ddd, $^3J(F,2')=23.5$, $^3J(F,4')=25.5$, $^2J(F,3')=53.5$ Hz, F-3′); IR (CH_2Cl_2): $\tilde{\nu}=2117.7$ cm^{-1} (N_3 st); HRMS (ESI$^+$ TOF): m/z: calcd for $C_{48}H_{40}F_1N_8O_2$: 779.3258; found: 779.3249 $[M+H]^+$.

3′-[(2-*N*-*tert*-Butoxycarbonyl-*O*-methyl)-L-tyrosyl]amido-2′,3′-dideoxy-2′-fluoro-6-*N*,5′-*O*-dityladenosine (10a): A mixture of Boc-L-Tyr(Me)-OH (106 mg, 0.36 mmol) and HOBt (48 mg, 0.36 mmol) was coevaporated from anhydrous THF. This solution (6 mL) was cooled down to 0°C under N_2 for 10 min then DIC (50 μL, 0.31 mmol) was added. After 10 min a solution of **9a** (200 mg, 0.257 mmol) and $(n\text{Bu})_3$P (146 μL, 0.59 mmol) in THF (5.4 mL) was added. The reaction mixture was stirred at RT overnight, then coevaporated with CH_2Cl_2 (40 mL), EtOAc (80 mL) were added. The organic phase was extracted with satd $NaHCO_3$ (40 mL) and H_2O (50 mL), dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo under reduced pressure. The residue was purified on a silica gel column (MePh/EtOAc 7:3) to give **10a** (249 mg, 94%) as a white amorphous solid. M.r. 115–124°C; $R_f=0.51$ (MePh/EtOAc 7:3); ^{1}H NMR ($CDCl_3$): $\delta=7.99$, 7.95 (2s, 2H, H-2, H-8), 7.50–7.16 (m, 30H, $6\times C_6H_5$), 6.96 (s, 1H, N^6-H), 6.89, 6.73 (2d, $J=8.6$ Hz, 4H, C_6H_4 Tyr), 6.17 (d, 1H, $^3J(1',F)=18.7$ Hz, H-1′), 6.00–5.84 (brs, 1H, NH amide), 5.36 (dd, 1H, $^3J(2',3')=4.5$, $^2J(2',F)=52.6$ Hz, H-2′), 5.11 (ddd, 1H, $^3J(3',2')=4.4$, $^3J(3',4')=9.9$, $^3J(3',F)=27.4$ Hz, H-3′), 5.04–4.95 (br, 1H, NH carbamate), 4.29–4.15 (brd, 1H, $J=6.8$ Hz, CH-α), 3.91–3.82 (m, 1H, H-4′), 3.75 (s, 3H, OCH_3), 3.46–3.37 (m, 2H, H-5′, H-5″), 2.95 (dd, 1H, $^3J(\beta_1,\alpha)=6.3$, $^2J(\beta_1,\beta_2)=13.8$ Hz, H-β$_1$), 2.74 (dd, 1H, $^3J(\beta_2,\alpha)=8.0$, $^2J(\beta_1,\beta_2)=13.8$ Hz, H-β$_2$), 1.40 ppm (s, 9H, $3\times CH_3$); ^{19}F NMR ($CDCl_3$): $\delta=-196.68$ ppm ("quint"=ddd, $^3J(F,3')=27.5$, $^3J(F,1')=18.7$, $^2J(F,2')=52.6$ Hz, F-2′); ^{13}C NMR ($CDCl_3$): $\delta=171.5$, 158.8, 155.7, 154.3, 152.7, 148.2, 145.1, 143.6, 138.2, 130.3, 129.2, 129.0, 128.5, 128.1, 127.4, 127.1, 121.6, 114.4, 94.2 (d, $^1J(F,C2')=185.1$ Hz), 88.3 (d, $^2J(F,C1')=34.5$ Hz), 87.3, 81.5 (d, $^3J(F,C4')<1$ Hz), 80.7, 71.6, 62.8, 56.1, 55.5, 50.2 (d, $^2J(F,C3')=16.2$ Hz), 37.7, 28.5 ppm; HRMS (FAB$^+$): m/z: calcd for $C_{63}H_{60}F_1N_7O_6$: 1029.4589; found: 1029.4584 $[M]^+$.

2′-[(2-*N*-*tert*-Butoxycarbonyl-*O*-methyl)-L-tyrosyl]amido-2′,3′-dideoxy-3′-fluoro-6-*N*,5′-*O*-dityladenosine (10b) and 2′-[(2-*N*-*tert*-butoxycarbonyl-*O*-methyl)-L-tyrosyl]triazeno-2′,3′-dideoxy-3′-fluoro-6-*N*,5′-*O*-dityladenosine (10d): Same procedure as for **10a** except that the reaction temperature remained at 0°C overnight. The extracted reaction mixture was purified on a silica gel column (MePh/EtOAc 8:2) to give the mixture of **10b** and **10d** as a white amorphous solid, ratio 78:22 according to ESI MS. $R_f=0.38$ for **10b** and 0.41 for **10d** (MePh/EtOAc 8:2); ^{1}H NMR ($CDCl_3$): $\delta=10.34$ (br, NH amide **10d**), 8.20, 8.07 (2s, 2H, H-2, H-8 **10b**), 7.91 (brs, H-2 **10d**), 7.88 (s, H-8 **10d**), 7.47–7.20 (m, 30H, $6\times C_6H_5$), 7.05 (s, 1H, N^6-H), 6.90, 6.60 (2d, $J=8.6$ Hz, 4H, C_6H_4 Tyr **10b**), 6.82–6.67 (m, 1.7H, C_6H_4 Tyr **10d** + NH amide **10d**), 6.08 (d, 1H, $^3J(1',2')=9.3$ Hz, H-1′), 5.70–5.39 (m, NH amide **10b** + **10d**), 5.43 (ddd, 1H, $^3J(2',1')=9.4$, $^3J(2',3')=4.2$, $^2J(2',F)=28.3$ Hz, H-2′), 5.08 (dd, 1H, $^3J(3',2')=4.1$, $^3J(3',F)=54.7$ Hz, H-3′), 4.90 (d, 1H, $^3J(NH,\alpha)=7.5$ Hz, NH carbamate), 4.59 (dt, $^3J(4',F)=25.3$, $^3J(4',5')=4.5$ Hz, H-4′ **10d**), 4.46 (dt, $^3J(4',F)=28.2$, $^3J(4',5'/5'')=3.3$ Hz, H-4′ **10b**), 4.35–4.26 (m, 1H, CH-α), 3.79, 3.78, 3.72 (3s, OCH_3 **10d**), 3.64 (s, 3H, OCH_3 **10b**), 3.59–3.37 (m, H-5′, H-5″ **10d**), 3.51 (dd, 1H, $^3J(5',4')=3.7$, $^2J(5',5'')=10.6$ Hz, H-5′ **10b**), 3.39 (dd, 1H, $^3J(5'',4')=3.2$, $^2J(5'',5')=10.6$ Hz, H-5″ **10b**), 3.16–2.64 (m, H-β$_1$, H-β$_2$ **10d**), 3.06–2.94 (m, 1H, H-β$_1$ **10b**), 2.88 (dd, 1H, $^3J(\beta_2,\alpha)=6.6$, $^2J(\beta_2,\beta_1)=13.9$ Hz, H-β$_2$ **10b**), 1.41 ppm (s, 9H, $3\times CH_3$); ^{19}F NMR ($CDCl_3$): $\delta=-191.73$ ("quint"=dt, $^2J(F,3')=54.3$, $^3J(F,2')=^3J(F,4')\approx28.5$ Hz, F-3′ **10b**), −194.00 ("quint"=ddd, $^2J(F,3')=50.2$, $^3J(F,2')=24.2$, $^3J(F,4')=25.7$ Hz, F-3′ **10d**), −195.48 ppm (brm, F-3′ **10d**); ESI$^+$ MS: m/z: calcd for $C_{63}H_{61}FN_7O_6$ (**10b**): 1030.5; found: 1030.3; calcd for $C_{63}H_{61}FN_9O_6$ (**10d**): 1058.5; found: 1058.2 $[M]^+$.

2′,3′-Dideoxy-2′-fluoro-3′-(*O*-methyltyrosyl)aminoadenosine (2): Compound **10a** (220 mg, 0.21 mmol) was dissolved in a mixture of CF_3COOH and $C_2H_4Cl_2$ (8.5:1.5, 3.2 mL) and kept at room temperature for 4 h. MeOH (10 mL) was added to the reaction mixture and concentrated in vacuo under reduced pression to a volume of ≈1 mL. After chromatography over silica gel (EtOAc 100% → EtOAc/MeOH 9:1 → EtOAc/MeOH/H_2O 9:1:0.2 → EtOAc/MeOH/H_2O 8:2:0.3) the fractions were combined, evaporated in vacuo then lyophilized to give **2** (91.3 mg) as a white amorphous solid (^{19}F NMR signal integral ratio 1:1.34). Compound **2** (91.3 mg) was dissolved in H_2O nanopure (13.5 mL) to obtain a concentration of ≈10 mM and acidified to pH 3.4 (100% salt), then lyophilized to obtain **2**·TFA (102.8 mg, 86%) as a white amorphous solid. M.r. 134–146°C; $R_f=0.37$ (EtOAc/MeOH/H_2O 8:2:0.3); ^{1}H NMR (CD_3OD): $\delta=8.47$, 8.23 (2s, 2H, H-2, H-8), 7.21–6.95 (2d, 4H, C_6H_4 Tyr), 6.35 (d, 1H, $^3J(1',F)=18.8$ Hz, H-1′), 5.44 (dd, 1H, $^3J(2',3')=4.4$, $^3J(2',F)=52.4$ Hz, H-2′), 5.02 (ddd, 1H, $^3J(3',F)=26.7$, $^3J(3',4')=9.7$, $^3J(3',2')=4.5$ Hz, H-3′), 4.09 (t, 1H, $^3J(\alpha,\beta)=7.5$ Hz, CH-α), 4.04 (td, 1H, $^3J(4',5')\approx^3J(4',5'')\approx3.2$, $^3J(4',3')=9.7$ Hz, H-4′), 3.85–3.76 (m, 1H, H-5′), 3.80 (s, 3H, OCH_3), 3.46 (dd, 1H, $^3J(5'',4')=3.4$, $^3J(5'',5')=12.6$ Hz, H-5″), 3.10, 3.08 ppm (2dd, 2H, $^2J(\beta_1,\beta_2)=13.8$, $^3J(\beta,\alpha)=7.5$ Hz, H-β$_1$, H-β$_2$); ^{19}F NMR (CD_3OD): $\delta=-196.48$ (ddd, $^3J(F,1')=19.0$, $^3J(F,3')=26.4$, $^2J(F,2')=52.5$ ppm, F-2′); ^{13}C NMR (CD_3OD): $\delta=170.5$, 160.8, 156.7, 153.0, 149.8, 141.2, 131.6, 127.2, 120.4, 115.5, 95.1 (d, $^1J(F,C2')=186.1$ Hz), 89.4 (d, $^2J(F,C1')=34.4$ Hz), 83.3 (d, $^3J(F,C4')<1$ Hz), 60.8, 55.8, 55.7, 50.9 (d, $^2J(F,C3')=16.8$ Hz), 37.9 ppm; HRMS (CI$^+$): m/z: calcd for $C_{20}H_{25}F_1N_7O_4$: 446.1952; found: 446.1953 $[M+H]^+$.

3′-Azido-2′,3′-dideoxy-2′-fluoroadenosine (11a): Compound **9a** (531 mg, 0.68 mmol) was treated with a mixture of $CF_3COOH/C_2H_4Cl_2$ (8.5:1.5, 8.8 mL). After 3 h the reaction mixture was worked up as described above and purified by chromatography over silica gel (EtOAc/MePh 7:3 → EtOAc/MeOH 9:1 → EtOAc/MePh/MeOH 9:0.5:0.5) to give **11a** (151 mg, 75%) as a white solid. M.p. 182–183°C; $R_f=0.33$ (MePh/EtOAc 1:9); ^{1}H NMR ([D$_6$]DMSO): $\delta=8.35$, 8.16 (2s, 2H, H-2, H-8), 7.39 (brs, 2H, NH_2), 6.31 (dd, 1H, $^3J(1',2')=2.1$, $^3J(1',F)=18.3$ Hz, H-1′), 5.81 (ddd, 1H, $^3J(2',1')=2.1$, $^3J(2',3')=4.5$, $^3J(2',F)=52.4$ Hz, H-2′), 5.41 (t, 1H, $^3J(OH,5'/5'')=5.6$ Hz, OH-5′), 4.72 (ddd, 1H, $^3J(3',2')=4.6$, $^3J(3',4')=7.9$, $^3J(3',F)=21.4$ Hz, H-3′), 4.12 (dt, 1H, $^3J(4',3')=7.8$, $^3J(4',5'/5'')=3.1$ Hz, H-4′), 3.77 (ddd, 1H, $^3J(5',OH)=5.6$, $^3J(5',4')=3.2$, $^2J(5',5'')=12.5$ Hz, H-5′), 3.63 ppm (ddd, 1H, $^3J(5'',OH)=6.0$, $^3J(5'',4')=3.6$, $^2J(5'',5')=12.5$ Hz, H-5″); ^{1}H NMR ($CDCl_3$): $\delta=8.33$, 7.86 (2s, 2H, H-2 and H-8); 6.50 (dd, 1H, $^3J(OH,5')=2.4$, $^3J(OH,5'')=11.8$, OH-5′); 6.09–5.89 (m, 2H, H-1′, H2′), 5.68 (brs, 2H, NH_2), 4.62–4.60 (m, 1H, H-3′), 4.24 (brt, 1H, H-4′), 3.96 (brdd, 1H, $^3J(5',OH)=1.7$, $^3J(5',5'')=13.3$ Hz, H-5′), 3.71 ppm (brtd, 1H, $^3J(5'',OH)=^3J(5'',5')=12.5$, $^3J(5'',4')=1.4$ Hz, H-5″); ^{19}F NMR ([D$_6$]DMSO): $\delta=-199.18$ (ddd, $^3J(F,3')=21.2$, $^3J(F,1')=18.7$, $^2J(F,2')=52.6$ ppm, F-2′); ^{19}F NMR ($CDCl_3$): $\delta=-207.0$–207.8 (m, F-2′); ^{13}C NMR (CD_3OD): $\delta=157.5$, 154.0, 150.0, 141.4, 120.7, 95.4 (d, $^1J(F,C2')=190.4$ Hz), 88.8 (d, $^2J(F,C1')=33.5$ Hz), 84.0 ('d', $^3J(F,C4')<1$ Hz), 61.8, 60.8 ppm (d, $^2J(F,C3')=15.2$ Hz); HRMS (EI$^+$): m/z: calcd for $C_{10}H_{11}F_1N_8O_2$: 294.0989; found: 294.0986 $[M]^+$.

2′-Azido-2′,3′-dideoxy-3′-fluoroadenosine (11b): The same procedure as for **9a** was applied to **9b** (90 mg, 0.12 mmol) in $CF_3COOH/C_2H_4Cl_2$ (8.5:1.5, 1.5 mL) to give after colmun chromatography **11b** (24 mg, 70%) as a white solid. M.p. 172–174°C; $R_f=0.27$ (MePh/EtOAc 1:9); ^{1}H NMR

($CDCl_3$): δ = 8.32, 7.84 (2s, 2H, H-2, H-8), 6.81 (dd, 1H, ^3J(OH,5′) = 2.4, ^3J(OH,5″) = 12.1 Hz, OH-5′), 5.90 (d, 1H, ^3J(1′,2′) = 8.7 Hz, H-1′), 5.70 (brs, 2H, NH_2), 5.21 (dd, 1H, ^3J(3′,2′) = 4.1, ^2J(3′,F) = 54.2 Hz, H-3′), 4.96 (ddd, 1H, ^3J(2′,3′) = 4.2, ^3J(2′,1′) = 8.7, ^3J(2′,F) = 26.3 Hz, H-2′), 4.56 (d"tW, 1H, ^3J(4′,F′) = 28.1, ^3J(4′,5′) ≈ 1.3 Hz, H-4′), 3.97 (ddd, 1H, ^3J(5′,F) = 3.2, ^3J(5′,4′) = 1.5, ^2J(5′,5″) = 13.2 Hz, H-5′), 3.81 ppm (brt, 1H, ^3J(5″,OH) = ^2J(5″,5′) = 12.4, ^3J(5″,4′) ≈ 1.5 Hz, H-5″); ^{19}F NMR ($CDCl_3$): δ = −194.27 ppm (dtd, ^4J(F,5′) = 3.0, ^3J(F,4′) ≈ ^3J(F,2′) ≈ 27, ^2J(F,3′) = 54.5 Hz, F-3′); ^{13}C NMR ($CDCl_3$): δ = 156.4, 152.9, 148.6, 140.7, 121.6, 93.9 (d, ^1J(F,C3′) = 184.8 Hz), 89.0, 86.7 (d, ^2J(F,C4′) = 21.6 Hz), 63.5 (d, ^2J(F,C2′) = 16.0 Hz), 62.6 ppm (d, ^3J(F,C5′) = 11.6 Hz); HRMS (EI$^+$): *m*/*z*: calcd for $C_{10}H_{11}F_1N_8O_2$: 294.0989; found: 294.0983 $[M]^+$.

***N,N*-Di-*n*-butylformamide dimethylacetal**: Di-*n*-butyl formamide (50 mL) and fresh dimethyl sulfate (26 mL) were mixed under an inert atmosphere and heated to reflux (100 °C) during 4 h, then cooled to ambient temperature and stirred over night. The mixture was worked up with ice-cold absolute MeOH (150 mL) into which sodium (8 g) had been dissolved before. After the temperature returned to ambient, the solvent was evaporated under reduced pressure, diethyl ether was added under vigorous stirring and the precipitate was filtered off and rinsed with more ether. The filtrate was evaporated under reduced pressure and the oily residue distilled in vacuo at up to 180 °C to give a clear colorless oil that could be safely stored under an inert atmosphere in the cold.

3′-Azido-6-*N*-(di-*n*-butylamino)methylene-2′,3′-dideoxy-2′-fluoroadenosine (12): Compound **11a** (150 mg, 0.51 mmol) was dissolved in anhydrous MeOH (2.6 mL) under N_2 and *N,N*-di-*n*-butylformamide dimethylacetal (414 mg, 2.04 mmol) was added. The solution was stirred at RT for 30 min followed by evaporation. The residue was purified by chromatography on silica gel (EtOAc/MePh 3:7 → EtOAc/MePh 5:5 → EtOAc/MePh 7:3 → EtOAc/MePh 8:2) to give **12** (206 mg, 93%) as a white amorphous solid. M.r. 99–111 °C; R_f = 0.54 (EtOAc/MePh 9:1); ^{1}H NMR ([D$_6$]DMSO): δ = 8.94 (s, 1H, N^6=CH), 8.47, 8.45 (2s, 2H, H-2, H8), 6.37 (dd, 1H, ^3J(1′,2′) < 1.5, ^3J(1′,F) = 18.4 Hz, H-1′), 5.82 (brddd, 1H, ^3J(2′,3′) ≈ 4.0, ^3J(2′,1′) < 1.5, ^3J(2′,F) = 52.3 Hz, H-2′), 5.40–5.37 (m, 1H, OH-5′), 4.73 (ddd, 1H, ^3J(3′,2′) = 4.4, ^3J(3′,4′) = 7.9, ^3J(3′,F) = 22.2 Hz, H-3′), 4.18–4.12 (m, 1H, H-4′), 3.83–3.58 (m, 4H, H-5′, H-5″, N(CH_2CH$_2$CH$_2$CH$_3$)), 3.46 (t, 2H, ^3J = 6.8 Hz, N(CH_2CH$_2$CH$_2$CH$_3$)), 1.67–1.54 (m, 4H, N(CH$_2$$CH_2CH_2CH_3$)$_2$), 1.37–1.27 (m, 4H, N(CH$_2$CH$_2$$CH_2CH_3$)$_2$), 0.96–0.90 ppm (m, 6H, N(CH$_2$CH$_2$CH$_2$$CH_3$)$_2$); ^{1}H NMR ($CDCl_3$): δ = 8.99 (s, 1H, N^6=CH), 8.49 (s, 1H, H-2), 7.92 (s, 1H, H-8), 6.59 (brd, 1H, ^3J(OH,5′) = 11.3 Hz, OH-5′), 6.10–5.90 (m, 2H, H-1′, H-2′), 4.64–4.60 (m, 1H, H-3′), 4.26–4.22 (m, 1H, H-4′), 3.98 (brd, 1H, ^3J(5′,OH) = 13.2 Hz, H-5′), 3.77–3.65 (m, 3H, H-5″, N(CH_2CH$_2$CH$_2$CH$_3$)), 3.41 (t, 2H, ^3J = 7.3 Hz, N(CH_2CH$_2$CH$_2$CH$_3$)), 1.70–1.60 (m, 4H, N(CH$_2$$CH_2CH_2CH_3$)$_2$), 1.44–1.31 (m, 4H, N(CH$_2$CH$_2$$CH_2CH_3$)$_2$), 0.99–0.9 ppm (m, 6H, N(CH$_2$CH$_2$CH$_2$$CH_3$)$_2$); ^{19}F NMR ([D$_6$]DMSO): δ = −198.93 ppm (ddd, ^3J(F,3′) = 21.9, ^3J(F,1′) = 18.6, ^2J(F,2′) = 52.4 Hz, F-2′); ^{19}F NMR ($CDCl_3$): δ = −207.2–208.0 ppm (m, F-2′); ^{13}C NMR ($CDCl_3$): δ = 160.7, 158.4, 152.2, 150.0, 141.2, 127.2, 92.1 (d, ^1J(F,C2′) = 196.3 Hz), 88.0 (d, ^2J(F,C1′) = 31.0 Hz), 84.7 (d, ^3J(F,C4′) = 2.0 Hz), 62.4, 61.0 (d, ^2J(F,C3′) = 14.2 Hz), 52.0, 45.3, 30.9, 29.2, 20.1, 19.7, 13.8, 13.6 ppm; HRMS (CI$^+$): *m*/*z*: calcd for $C_{19}H_{29}F_1N_9O_2$: 434.2428; found: 434.2421 $[M+H]^+$.

4-*N*-Acetyl-2′-*O*-triisopropylsilyloxymethyl-5′-*O*-dimethoxytritylcytidyl-{3′-*O*^{P}-2-cyanoethyl}-5′-[3′-azido-2′,3′-dideoxy-2′-fluoro-6-*N*-(di-*n*-butylamino)methylene]adenylate (13): Compound **12** (90 mg, 0.208 mmol) and ethylthiotetrazole (40 mg, 0.312 mmol) were co-evaporated with anhydrous CH_3CN (3 × 5 mL) under reduced pressure and redissolved in anhydrous CH_3CN (0.7 mL). After addition of commercial N^4-acetyl-5′-*O*-dimethoxytrityl-2′-*O*-triisopropylsilyloxymethylcytid-3′-yl-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (425 mg, 0.437 mmol) in anhydrous CH_3CN (0.8 mL), the solution was stirred at RT for 15 min followed by addition of a solution of 0.2 M I_2/THF/pyridine/H_2O (1.15 mL, 0.229 mmol I_2), faint yellow color persisted at the end. The reaction mixture was concentrated in vacuo under reduced pressure to one half the volume, taken up in EtOAc (36 mL), and extracted with 0.2 M $NaHSO_3$ (2 × 8 mL) and saturated NaCl (5 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo under reduced pressure. The residue was purified on a silica gel column (EtOAc/MePh 8:2 → EtOAc/MePh 9:1 → EtOAc/MeOH 9.5:0.5) to give **13** (253 mg, 92%) as a white amorphous solid. M.r. 97–105 °C; R_f = 0.36 (CH_2Cl_2/CH_3OH 95:5); ^{1}H NMR ($CDCl_3$): δ = 9.02–8.85 (brd, 1H, N^{4}H-Ac, diast), 8.96 (s, 1H, N^6=CH), 8.49 (s, 1H, H-2), 8.35, 8.28 (2d, 1H, ^3J(6,5) = 7.7 Hz, H-C6, diast), 8.02, 7.90 (2s, 1H, H-8, diast), 7.38–6.83 (m, 13H, CH arom DMT), 7.07, 7.02 (2d, 1H, ^3J(5,6) = 7.7, H-C5, diast), 6.20, 6.14 (2d, 1H, ^3J(1′,2′) < 1 Hz, H-1′Cyt, diast), 6.17, 6.11 (2d, 1H, ^3J(1′,2′) < 1.5, ^3J(1′,F) = 20.7 Hz, H-1′ Ade, diast), 5.80, 5.75 (2ddd, 1H, ^3J(2′,1′) < 1.5, ^3J(2′,3′) = 4.6, ^2J(2′,F) = 52.5 Hz, H-2′ Ade, diast), 5.25 (dd, 1H, ^3J = 4.5, ^2J = 10.0 Hz, CH_2OSi), 5.16 (t, 1H, ^3J = 4.4 Hz, CH_2OSi), 5.06–4.93 (m, 1H, H-3′ Cyt), 4.79–4.66 (m, 1H, H-3′ Ade), 4.64–4.59 (m, 1H, H-2′ Cyt), 4.55–4.49 (m, 1H, H-5′ Ade), 4.38–4.32 (m, 2H, H-4′ Cyt + H-5″ Ade), 4.26–4.17 (m, 2H, H-4′ Ade + CH_2-O cyanoethyl), 3.97–3.84 (m, 1H, CH_2-O cyanoethyl), 3.80–3.78 (4s, 6H, 2 × O-CH_3 DMT), 3.71–3.63 (m, 3H, H-5′ Cyt + N(CH_2CH$_2$CH$_2$CH$_3$)), 3.46–3.35 (m, 3H, H-5″ Cyt + N(CH_2CH$_2$CH$_2$CH$_3$)), 2.67 (dt, 1H, CH_2CN), 2.47 (dt, 1H, CH_2CN), 2.15, 2.10 (2s, 3H, CH_3-Ac), 1.67–1.60 (m, 4H, N(CH$_2$$CH_2CH_2CH_3$)$_2$), 1.41–1.31 (m, 4H, N(CH$_2$CH$_2$$CH_2CH_3$)$_2$), 1.02–0.82 ppm (m, 30H, N(CH$_2$CH$_2$CH$_2$$CH_3$)$_2$ + Si(CH(CH_3)$_2$)$_3$ + Si(CH(CH$_3$)$_2$)$_3$); ^{19}F NMR ($CDCl_3$): δ = −196.93 (dt, ^3J(F,3′) = ^3J(F,1′) ≈ 21.5, ^2J(F,2′) = 52.6 Hz, F-2′, diast), −197.3– −198.3 ppm (brm, F-2′, diast); ^{31}P NMR (^{1}H decoupled, $CDCl_3$): δ = −1.39, −1.51 ppm (2s, diast); ^{13}C NMR ($CDCl_3$): δ = 171.12, 171.07 (diast); 163.11, 163.07 (diast); 160.29, 160.23 (diast); 158.79, 158.76 (diast); 158.4, 154.87, 154.75 (diast); 152.9; 150.8; 150.6; 144.53, 144.43 (diast); 143.86, 143.82 (diast); 140.43, 140.39 (diast); 135.15, 135.08, 135.03, 134.99 (diast); 130.17, 130.10 (diast); 128.32, 128.25 (diast); 128.1; 127.31, 127.22 (diast); 126.33, 126.24 (diast); 116.73, 116.17 (diast); 113.4; 96.75, 96.49 (diast); 93.84, 93.67 (d diast, ^1J(F,C2′) = 191.0 Hz); 90.25, 90.00 (diast); 88.67, 87.42 (diast); 88.51, 87.64 (d diast, ^2J(F,C1′) = 33.1 Hz); 81.10, 80.91 (diast); 79.52, 79.41 (diast); 79.26, 79.15 (diast); 78.32, 78.23 (diast, ^3J(F,C4′) < 1 Hz); 73.67, 73.19 (diast); 66.65, 66.26 (diast); 62.87, 62.30 (diast); 60.80, 60.28 (diast); 59.35, 59.16 (d diast, ^2J(F,C3′) = 14.9 Hz); 55.28, 55.24 (diast); 52.0; 45.3; 30.9; 29.2; 24.84, 24.80 (diast); 20.2; 19.8; 19.36, 19.26 (d diast); 17.81, 17.77 (diast); 13.9; 13.7); 11.89, 11.89 ppm (diast); IR (CH_2Cl_2): $\tilde{\nu}$ = 2114.1 cm^{-1} (N_3 st); HRMS (ESI$^+$ TOF): *m*/*z*: calcd for $C_{64}H_{86}F_1N_{13}O_{13}P_1Si_1$: 1322.5959; found: 1322.5908 $[M+H]^+$.

4-*N*-Acetyl-5′-*O*-dimethoxytrityl-2′-*O*-triisopropylsilyloxymethylcytid-3′-yl-{2-cyanoethyl}-5′-[2′,3′-dideoxy-3′-(*N*-9-fluorenylmethoxycarbonyl-*O*-methyl-L-tyrosyl)amino-2′-fluoro-6-*N*-(di-*n*butylamino)methylene]adenylate (14a): A mixture of Fmoc-L-Tyr(Me)-OH (49 mg, 0.12 mmol) and HOBt (16 mg, 0.12 mmol) was coevaporated twice from anhydrous THF. A THF solution (2 mL) thereof was cooled down to 0 °C under N_2 for 10 min then DIC (16 μL, 0.10 mmol) was added. After 10 min a solution of **13** (110 mg, 0.08 mmol) and (nBu)$_3$P (47 μL, 0.19 mmol) in THF (2 mL), kept at RT, was added and the reaction mixture was stirred at RT overnight. The reaction mixture was coevaporated with CH_2Cl_2 (10 mL) and then added to EtOAc (28 mL), extracted with satur. $NaHCO_3$ (14 mL) and H_2O (2 × 10 mL), dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo under reduced pressure. Cyclohexane (≈50 mL) was added and the precipitate filtered and rinsed with more cyclohexane. The residue (a white powder now essentially free of nBu$_3$PO) was redissolved in a minimum amount of EtOAc/MeOH 95:5 and purified on a silica gel column (EtOAc/MePh 95:5 → EtOAc → EtOAc/MeOH 95:5) to give **14a** (131 mg, 94%) as a white amorphous solid. M.r. 106–110 °C; R_f = 0.39 (CH_2Cl_2/CH_3OH 95:5); ^{1}H NMR ($CDCl_3$): δ = 8.97 (s, 1H, N^6=CH), 8.62 (br, 1H, NH-acetyl), 8.56 (s, 1H, H-2), 7.79–7.39 (m, 8H, CH arom Fmoc), 7.71 (s, 1H, H-8), 7.66–7.61 (2d, 1H, H-C6 Cyt, diast), 7.34–6.79 (m, 14H, CH arom DMT + NH amide), 7.20–7.17 (2d, 1H, H-C5 Cyt, diast), 6.96–6.69 (2d, 4H, C_6H_4 Tyr), 6.24–5.83 (m, H-1′ Cyt + H-1′ Ade), 5.68–5.34 (m, H-2′ Ade + H-3′ Ade + NH carbamate), 5.22–5.17 (m, 2H, CH_2OSi), 4.93–4.91 (m, 1H, H-3′ Cyt), 4.63–4.55 (m, 1H, H-2′ Cyt), 4.49–4.41 (m, 1H, H-5′ Ade), 4.32–4.20 (m, 4H, CH_2O Fmoc + H-4′ Cyt + H5″ Ade), 4.06–3.93 (m, 2H, H-4′ Ade + CH-α), 3.81–3.78 (m, 2H, CH_2O cyanoethyl), 3.74–3.71 (m, 10H, H-5′ Cyt + H-9″ Fmoc + 2 × O-CH_3 DMT + N(CH_2CH$_2$CH$_2$CH$_3$)), 3.49 (s, 3H, OCH_3 Tyr), 3.40 (t, 2H, N(CH_2CH$_2$CH$_2$CH$_3$)$_2$), 3.35–3.29 (m, 1H, H5″ Cyt), 3.00–2.87 (m, 2H,

CH_2-β_1,β_2), 2.59–2.05 (dt, 2H, CH_2CN, diast), 2.03 (s, 3H, CH_3-Ac), 1.72–1.56 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$), 1.41–1.29 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$), 1.03–0.88 ppm (m, 30H, $N(CH_2CH_2CH_2CH_3)_2$ + $Si(CH(CH_3)_2)_3$ + $Si(CH(CH_3)_2)_3$); ^{19}F NMR ($CDCl_3$): $\delta = -194.7$ (dt, ^3J(F,3′) = ^3J(F,1′) = 21.5, ^2J(F,2′) = 52.7 Hz, F-2′, diast); −194.8–−196.0 ppm (2 br m, F-2′, diast/aggreg); ^{31}P NMR (^{1}H decoupled, $CDCl_3$): $\delta = -1.45$, −1.50 ppm (2 s, diast); ^{13}C NMR ($CDCl_3$): $\delta = 172.7$; 171.4; 162.87, 162.81 (diast); 160.4; 158.9, 158.7; 158.6, 158.4; 156.22, 156.17 (diast); 153.21, 153.16 (diast); 152.8; 150.92, 150.83 (diast); 149.51, 149.45 (diast); 144.1; 143.8; 143.7; 141.4; 141.4; 135.7, 135.2; 130.7, 130.6; 130.3, 130.2; 128.3, 128.2; 128.0, 127.9; 127.4; 127.3, 127.2; 126.76, 126.49 (diast); 125.11, 125.07; 120.19, 120.10; 116.66, 116.31 (diast); 114.0, 113.7; 113.5; 113.4; 97.81, 97.65 (diast); 93.6 (d, ^1J(F,C2′) = 191.0 Hz); 89.43, 89.16 (diast); 88.6 (d, ^2J(F,C1′) = 35.6 Hz); 87.6; 82.55, 82.43 (diast); 78.6; 78.0; 77.8 (^3J(F,4′) < 1 Hz); 77.4; 67.4; 67.36, 67.24 (diast); 63.82, 63.78 (diast); 62.71, 62.66 (diast); 55.8 (d, ^2J(F,3′) = 16.8 Hz); 55.4, 55.3; 55.21, 55.14; 52.0; 47.1; 45.3; 45.3; 31.1; 29.4; 24.8; 20.3; 19.9; 19.41, 19.34 (diast); 17.8; 14.1; 13.8; 11.9 ppm; HRMS (ESI$^+$ TOF): m/z: calcd for $C_{89}H_{109}F_1N_{12}O_{17}P_1Si_1$: 1695.7525; found: 1695.7469 $[M+H]^+$.

5′-*O*-Dimethoxytritylcytidylyl-{3′-*O*^{P}}-5′-[2′,3′-dideoxy-2′-fluoro-3′-(*O*-methyl-L-tyrosyl)amino]adenylate (15): Compound **14a** (71 mg, 0.04 mmol) was dissolved in 33% CH_3NH_2/EtOH (10 mL). The solution was stirred at RT in a closed vessel for 35 min, then evaporated in vacuo under reduced pressure and co-evaported with THF (2 × 5 mL), followed by addition of 1 M TBAF/THF (252 μL, 0.252 mmol) and THF (0.5 mL). After 40 min the reaction mixture was concentrated in vacuo under reduced pressure. The chromatography column was conditioned with EtOAc/MeOH/H_2O 9:2:1 and the mixture was purified by chromatography (EtOAc/MeOH/H_2O 9:2:1 → EtOAc/MeOH/H_2O 8:3:1 → EtOAc/MeOH/H_2O 8:4:1) to give **15**·TBA (45 mg, 83%) as a white amorphous solid (as the mono-tetrabutylammonium salt, as determined by ^{13}C NMR and quantified by ^{1}H NMR). M.r. 174–180 °C; $R_f = 0.35$ (EtOAc/MeOH/H_2O 8:4:1); ^{1}H NMR (CD_3OD): $\delta = 8.24$, 8.15 (2 s, 2H, H-2, H-8), 7.91 (d, 1H, ^3J(6,5) = 7.5 Hz, H-C6 Cyt), 7.41–6.82 (m, 17H, 13 × CH arom DMT + 4H, C_6H_4 Tyr), 6.22 (d, 1H, ^3J(1′,F) = 19.0 Hz, H-1′ Ade), 6.02 (d, 1H, ^3J(1′,2′) = 4.1 Hz, H-1′ Cyt), 5.49–5.28 (m, 2H, H-C5 Cyt + H-2′ Ade), 5.04–4.91 (ddd + m, 2H, H-3′ Ade + H-3′ Cyt), 4.43 (t, 1H, ^3J(2′,1′) = 4.5 Hz, H-2′ Cyt), 4.33–3.99 (m, 4H, H-4′ Ade + H-4′ Cyt + H5′,H5″ Cyt), 3.78–3.68 (m, 10H, 2 × OCH_3 DMT + OCH_3 Tyr + CH-α), 3.51–3.35 (m, 2H, H5′ + H5″ Ade), 3.26–3.21 (m, 8H, $(CH_3CH_2CH_2CH_2)_4N^+$), 3.01 (dd, 1H, $^3J(\beta_1,\alpha) = 6.0$, $^2J(\beta_1,\beta_2) = 13.8$ Hz, H-β_1), 2.80 (dd, 1H, $^3J(\beta_2,\alpha) = 7.7$, $^2J(\beta_1,\beta_2) = 13.8$ Hz, H-β_2), 1.71–1.61 (m, 8H, $(CH_3CH_2CH_2CH_2)_4N^+$), 1.42 (sext, $J = 7.5$ Hz, 8H, $(CH_3CH_2CH_2CH_2)_4N^+$), 1.02 ppm (t, 12H, $(CH_3CH_2CH_2CH_2)_4N^+$); ^{19}F NMR (CD_3OD): $\delta = -197.57$ ppm (ddd, ^3J(F,3′) = 26.2, ^3J(F,1′) = 19.2, ^2J(F,2′) = 52.5 Hz, F-2′); ^{31}P NMR (CD_3OD): $\delta = 0.00$ ppm (q, $^3J = 6.5$ Hz); ^{13}C NMR (D_2O/trace CD_3OD): $\delta = 175.6$, 170.3, 167.4, 160.2, 158.4, 157.3, 154.0, 150.0, 146.0, 142.6, 140.9, 136.8, 136.5, 131.5, 131.5, 129.7, 129.5, 128.9, 128.0, 120.5, 115.2, 114.2, 96.0, 95.0 (d, ^1J(F,C2′) = 186.3 Hz), 90.8, 88.3, 89.4 (d, ^2J(F,C1′) = 35.3 Hz), 83.4, 81.9 (s, ^3J(F,C4′) < 1 Hz), 76.1, 75.3, 66.0, 63.6, 59.5, 57.2, 55.7, 54.8, 52.0 (d, ^2J(F,C3′) = 16.0 Hz), 40.6, 24.8, 20.7, 14.0 ppm; HRMS (ESI$^-$ TOF): m/z: calcd for $C_{50}H_{53}F_1N_{10}O_{13}P_1$: −1051.3515; found: −1051.3625 $[M-H]^-$.

Cytidyl-{3′-*O*^{P}}-5′-[2′,3′dideoxy-2′-fluoro-3′-(*O*-methyl-L-tyrosyl)amino]-adenylate (3): Compound **15**·TBA (40 mg, 0.03 mmol) was dissolved in AcOH/H_2O 8:2 (15 mL). The solution was stirred at RT for 20 min and then lyophilised. The residue was purified by chromatography over a silica gel column which was conditioned and eluted with EtOAc/MeOH/H_2O 7:3:2 to give, after lyophilisation from H_2O, **3** (19 mg, 82%) as a white amorphous solid. M.r. 192–197 °C; $R_f = 0.31$ (EtOAc/MeOH/H_2O 7:3:2); ^{1}H NMR (D_2O): $\delta = 8.39$, 8.26 (2 s, 2H, H-2, H-8), 7.72 (d, 1H, ^3J(6,5) = 7.6 Hz, H-C6 Cyt), 7.22, 7.03 (2 d, 4H, C_6H_4 Tyr), 6.39 (d, 1H, ^3J(1′,F) = 17.2 Hz, H-1′ Ade), 5.75 (d, 1H, ^3J(5,6) = 7.6 Hz, H-C5 Cyt), 5.65 (d, 1H, ^3J(1′,2′) = 2.6 Hz, H-1′ Cyt), 5.26 (dd, 1H, ^3J(2′,3′) = 4.2, ^3J(2′,F) = 51.6 Hz, H-2′ Ade), 4.89 (ddd, 1H, ^3J(3′,F) = 28.5, ^3J(3′,2′) = 4.2 Hz, H-3′ Ade), 4.41–4.37 (m, 1H, H-3′ Cyt), 4.32–4.30 (m, 1H, H-2′ Cyt), 4.23–4.20 (m, 2H, H-4′ Cyt + H5′ Cyt), 4.11–4.09 (m, 1H, H-4′ Ade), 3.93–3.88 (m, 2H, CH-α + H5′ Ade), 3.85 (s, 3H, OCH_3 Tyr), 3.82–3.77 (m, 2H, H5″ Ade + H5″ Cyt), 3.08 (dd, 1H, $^3J(\beta_1,\alpha) = 6.1$, $^2J(\beta_1,\beta_2) = 13.7$ Hz, H-β_1), 2.95 ppm (dd, 1H, $^3J(\beta_2,\alpha) = 8.5$, $^2J(\beta_1,\beta_2) = 13.7$ Hz, H-β_2); ^{19}F NMR (CD_3OD): $\delta = -196.7$ ppm (ddd, ^3J(F,1′) = 18.4, ^3J(F,3′) = 26.3, ^2J(F,2′) = 52.4 Hz, F-2′); ^{31}P NMR (^{1}H decoupled, CD_3OD): $\delta = 0.00$; ^{13}C NMR (D_2O/trace CD_3OD): $\delta = 174.4$, 166.6, 159.1, 157.9, 156.4, 153.9, 148.8, 141.6, 139.8, 131.5, 128.7, 119.6, 115.3, 96.5, 94.7 (d, ^1J(F,C2′) = 186.1 Hz), 91.6, 88.1 (d, ^2J(F,C1′) = 35.4 Hz), 83.2, 80.7 (^3J(F,C4′) < 1 Hz), 74.2, 73.2, 63.8, 60.9, 56.4, 56.3, 49.8 (d, ^2J(F,C3′) = 16.6 Hz), 38.9 ppm; HRMS (ESI$^+$ TOF): m/z: calcd for $C_{29}H_{37}F_1N_{10}O_{11}P_1$: 751.2365; found: 751.2370 $[M+H]^+$.

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[22] Only very recently we learned that in the laboratories of Mikhailopulo and his collegues another synthetic route was pursued to synthesize, among a number of similar fluorinated compounds, azide **11a** (Scheme 3) and puromycine analogue **2** (Figure 1), in order to study the influence of various substituents that replace the 2′- and/or 3′-hydroxy groups of nucleosides on the equilibria between two furanose puckers, and structural informations thereof, using a maximum number of measured vicinal coupling constants as an input for the program PSEUROT v6.3. The regioisomer **11b** was already synthesized and analyzed by the authors (their compound **20**)[23] along with other 3′-deoxyfluororibonucleosides. Here we wish to report in this context on the approximate positions of the rapidly interconverting North–South equilibrium of β-hydroxyazides **8a** and **8b**, β-fluoroazides **9a**, **9b**, **11a**, **11b**, **12**, and **13**, as well as for the puromycine analogues (carboxamido derivatives) **10a**, **10b**, **2**, **14a**, **15** and **3**, as revealed by their scalar vicinal proton–proton coupling constants between H1′ and H2′, as well as vicinal fluor–proton coupling constants. Instead of analyzing our NMR data through PSEUROT, we should like to apply a slightly modified version of the well known and quite simple but robust model, the "10 Hertz rule-of-thumb", to estimate the pucker preference of our compounds in a given solvent. According to this rule, the ^3J(H1′α,H2′β) values—Hα for C5′-*trans*, Hβ for C5′-*cis* configured hydrogen atoms—indicate an approximate tendency for either a North- or South-type pucker preference of some pentafuranoses. Pure North-type ribo- or xylofuranose puckers (2′-pseudoaxial substituent) give rise to a zero coupling between H1′α and H2′β, pure South-type ribo- or xylofuranose puckers (2′-pseudoequatorial substituent) result in close to 10 Hertz coupling constants between H1′α and H2′β. Thanks to the above-mentioned study by Mikhailopulo et al.[23] we could correlate thirteen of their ^3J(H1′α,H2′β) values of eleven ribofuranose derivatives with their corresponding South preferences as determined through PSEUROT (their compounds **2**, **4**, **5**, **10b** in DMSO, **10b** in methanol, **11b** in DMSO, **11b** in methanol, **12b**, **17**, **20**, **23**, **24** and **25**). The linear correlation coefficient was $r=0.9938$ and the correlation showed that, although not overwhelmingly convincing due to the lack of very-high-North compounds, 0% South indeed corresponds to not very much above 0 Hz for ^3J(H1′α,H2′β) but the upper limit value for 100% South is more likely to correspond to 9.5 rather than 10 Hz (cf. Supporting Information), with an error margin of probably ±5%. The way we calculate from ^3J(H1′α,H2′β) values the pucker preference is thus more of a "9.5 Hertz rule-of-thumb": we thus multiply the ^3J(H1′α,H2′β) value in Hertz with 10.53 to obtain % South (=100 − % North). Of course this rule will not apply to ^3J(H1′α, H2′α) values (arabinofuranoses) or ^3J(H1′β, H2′) values (α-anomeric nucleoside derivatives). A close inspection of ^3J(H1′,H2′) of our compounds revealed a thus far unidentified pucker preference for the 3′-azido-3′-deoxyarabinofuranosyl derivative **8a** in $CDCl_3$ (^3J(H1′α, H2′α)=5.3 Hz), a ≈77% preference for the North-type pucker in 2′-azido-2′-deoxyxylofuranosyl derivative **8b** in $CDCl_3$ (^3J(H1′α,H2′β)=2.1 Hz), consistently and expectedly high North preferences for the 3′-azido-2′,3′-dideoxy-2′-fluororibofuranosyl derivatives **9a** (^3J(H1′α,H2′β)=1.3 Hz or ≈86% North in $CDCl_3$), **11a** (^3J(H1′α,H2′β)=2.1 Hz or ≈78% North in [D$_6$]DMSO), **12** (^3J(H1′α,H2′β) < 1.3 Hz or > 86% North in [D$_6$]DMSO) and **13** (^3J(H1′α,H2′β) < 1.5 Hz or >84% North in $CDCl_3$), consistently high South preferences for the regioisomeric 2′-azido-2′,3′-dideoxy-3′-fluororibofuranosyl derivatives **9b** (^3J(H1′α,H2′β)=7.9 Hz or ≈83% South in $CDCl_3$) and **11b** (^3J(H1′α,H2′β)=8.7 Hz or ≈92% South in $CDCl_3$, in agreement with ref. [23]), a quasi absolute South preference for the regioisomeric puromycine analogue **10b** (^3J(H1′α, H2′β)=9.3 Hz or ≈98% South in $CDCl_3$, the largest ^3J(H1′,H2′) measured[23]), and of course an absolute North preference (no measurable H1′α-H2′β coupling) for all 2′-deoxy-2′-fluoropuromycine analogues (3′-carboxamido derivatives) **10a**, **2**, **14a**, **15** and **3**, irrespective of the solvent ($CDCl_3$, CD_3OD or D_2O at pD 7). The apparent pucker preferences of all above furanosyl derivatives appear in agreement with Altona and Sundaralingam's[24] and Chattopadhyaya's,[25] as well as Brunck and Weinhold's[26] models of the dependence on the electron density depleting potency (quantifyable through Mullay's[27] or other empirical group electronegativities, see refs. [14] in [28]) of the 2′ and 3′ substituents through a subtle balance between stereoelectronic *gauche* versus anomeric effects[25] or the antiperiplanar effect[26] that govern the pucker equilibria of nucleosides (see discussion in ref. [23]). According to our measurements, the azido group seems, perhaps astonishingly, slightly more electronegative (more effective in electron density depletion) than a carboxamide function—albeit much weaker than hydroxyl—irrespective of a positive charge present or absent in the α-position of the amido substituent. In addition, we observed in the ^{19}F NMR spectra of the 3′-azido-2′,3′-dideoxy-2′-fluoro analogues **11a** and **12**—both bearing free hydroxyls in the 5′ position—a marked solvent dependence of the furanose pucker preference depending on whether the spectrum was measured in [D$_6$]DMSO (≈78 and >86% North, respectively) or in $CDCl_3$. In dimethyl sulfoxide we observed the usual ddd signal (as in all the other 3′-azido-2′,3′-dideoxy-2′-fluoro derivatives) at δ −199.2 and −198.9 ppm, respectively, which originate from three distinct scalar F,H coupling constants, one geminal of ^2J(F,H) ≈52.5 Hz (F2′–H2′) and two different vicinals of ^3J(F,H) ≈18.6 Hz for F2′–H1′ and ^3J(F,H)=21–22 Hz for F2′–H3′ (in the ^{1}H NMR spectra 52.4, 18.3 and 21.4 Hz, respectively). Were the puckers of **11a** and **12** in DMSO 100% North, these ^3J(F,H) values would represent torsional angles, according to crystal structure coordinates of several nucleos-(t)ide North puckers, of between −14 and −33° (not too far from *syn*) for H1′α-C1′-C2′-F2′α and 162–163° (close to *anti*) for H3′β-C3′-C2′-F2′α. In chloroform the multiplicity of the ^{19}F NMR signals of **11a** and **12** collapsed each to a kind of dd signal—with more minor badly or unresolved peaks within—and shifted to around −207.7 ppm (which folded in to +58.5 ppm when the usual limit of ^{19}F high field detection of −206.5 ppm was applied, cf. Supporting Information). These pseudo-dd signals did not show first-order multiplicity: the "roof effect" (order of peak intensities) seemed inversed—as if the downfield half of the resonance were folded in from an even higher field—and the measured peak distances did not reveal the true F–H coupling constants, since the apparent peak splittings (in Hz) could not be found in the corresponding ^{1}H NMR spectra in $CDCl_3$ (cf. Supporting Information). In the latter, however, the absence of a vicinal F2′–H3′ coupling in the H3′ signals and a 1.2 ppm downfield shift of the 5′-OH signals with respect to the ^{1}H NMR spectra in [D$_6$]DMSO became evident, along with other differences. Unfortunately, the H1′–H2′ coupling constants could not be elucidated with certainty owing to the close proximity of those resonances in $CDCl_3$. We interpret the unusual ^{19}F NMR signals and

the strong 5′-OH downfield shift in the ^{1}H NMR spectra of **11a** and **12** in $CDCl_3$ with a stable intramolecular 5′-OH···N3 hydrogen bridge that forces the base into the usually less favored *syn* conformation and the furanose pucker into one belonging to the South type (despite F2′α) where the torsional angle H3′β-C3′-C2′-F2′α is assumed to be close to perpendicular (≈86°, as for H3′β-C3′-C2′-O2′α in the crystal structure ACADOS of 3′-*O*-acetyladenosine).[29] Apparently, the forced North-to-South rearrangement, thus, the repositioning of F2′α from the usually preferred pseudoaxial into the pseudoequatorial orientation through an intramolecular hydrogen bond, caused enhanced shielding, an upfield shift of the corresponding ^{19}F resonance of 8.6 to 8.7 ppm. Not unexpectedly, the intramolecular hydrogen bond seems to be preferred in chloroform but disfavored in dimethyl sulfoxide. An estimated 3 to 5 kcal mol^{-1} free energy drop due to the formation of 5′-OH···N3 gives us an upper limit for the compensated for rise in free energy due to the pseudoequatorial orientation of a fluorine substituent, which is in accord with recent measurements and calculations of its stereoelectronic *gauche* effect (in general 1–2 kcal mol^{-1} in favor of *gauche* versus *anti* F-C-C-N).[13] We refrain, however, from quantifying the energetics of *gauche* (maximal at ±60°) or anomeric effects (optimal at 0 and 180°) in geometries where torsional angles between heteroatoms of 90° (O4′-C1′-C2′-O2′α), 43° (O3′α-C3′-C2′-O2′α) and −151° (N9β-C1′-C2′-O2′α) dominate the scene of North puckers (taken from CUPYUH),[30] while in typical South puckers the respective torsional angles of 161.5, −36 and 80° prevail (ACADOS).[29] We observed similar but less marked differences between the ^{19}F NMR spectra of the regioisomeric 3′-deoxyfluoro analogues **9b** (5′-trityl ether, ≈83% South), **10b** (5′-trityl ether, ≈98% South) and **11b** (5′-OH, ≈92% South) all in $CDCl_3$ (cf. Supporting Information). Azide **9b** showed quite clearly a fairly resolved ddd multiplicity of the F3′-signal at −195.1 ppm, which originates from three distinct scalar F,H coupling constants, one geminal of ^2J(F,H) = 53.5 Hz for F3′–H3′ and two different vicinals of ^3J(F,H) = 23.5 Hz for F3′-H2′ and 25.5 Hz for F3′-H4′. Amide **10b** showed, like all amides, a quintuplet of the F3′ signal at −191.7 ppm, which originates from two distinct scalar F,H coupling constants, one geminal of twice the value of one and the same vicinal of ^3J(F,H) = 28.2 Hz for F3′–H2′ and F3′–H4′ (in the ^{1}H NMR spectrum: ^2J(H3′,F3′) = 54.8 Hz, ^3J(H2′,F3′) = 28.2 Hz and ^3J(H2′,F3′) = 28.6 Hz). These ^3J(F,H) values represent torsional angles, according to crystal structure coordinates of several nucleos(t)ide South puckers, of between −154 and −180° (close to *anti*) for H2′β-C2′-C3′-F3′α and 13–34° (close to *syn*) for H4′α-C4′-C3′-F3′α. The ^{19}F NMR resonance of **11b**, in contrast, showed up as a dtd signal at −194.2 ppm which originates from three distinct scalar F,H-coupling constants, one geminal of ^2J(F,H) = 54.6 Hz for F3′-H3′ (in the ^{1}H NMR spectrum 54.2 Hz), one and the same vicinal coupling constant of ^3J(F,H) ≈27 Hz for F3′–H2′ and F3′–H4′ (in the ^{1}H NMR spectrum 26.3 and 28.1 Hz, respectively), as well as an exceptional long range coupling of ^4J(F,H) = 3.0 Hz for F3′-H5′ (not F′-H5″, as revealed by H–D exchange in the ^{1}H NMR spectrum, cf. Supporting Information)! The ^{1}H resonance of 5′-OH shifted even more to lower fields and appeared as a clearly resolved dd signal at 6.8 ppm (6.6 ppm for 5′-OH of **11a** and **12** in $CDCl_3$). Again, we interpret the additional F3′–H5′ long range coupling in the ^{19}F and ^{1}H resonances (the only observed) and the strong 5′-OH downfield shift in the ^{1}H NMR spectrum of **11b** in $CDCl_3$ with a stable 5′-OH···N3 hydrogen bridge impossible for **9b** or **10b**. Since **9b**, **10b** and **11b** all favor the pseudoaxial orientation of F3′α, thus, the South pucker, no large upfield shift of the ^{19}F resonance upon formation of the intramolecular hydrogen bond is expected. On the contrary, the ^{19}F resonance of azide **11b** shifted downfield by 1.1 ppm with respect to azide **9b** because its South pucker, thus, the pseudoaxial orientation of F3′α is even higher populated than in **9b**, as confirmed by a larger H1′–H2′ coupling constant in **11b** (8.7 Hz) than in **9b** (7.9 Hz). In conclusion, not only are chemical shifts of ^{19}F resonances extremely sensitive to hydrogen bonding elsewhere in the molecule[31] and to conformational changes. For instance, our observation from the analysis of vicinal proton–proton coupling constants ^3J(H1′α,H2′β) that are larger for the 2′-fluoro-3′-azides than for the corresponding 2′-fluoro-3′-amides and smaller for the 3′-fluoro-2′-azide than for the 3′-fluoro-2′-amide, therefore, suggesting a weaker preference for the pseudoaxial orientation of fluorine in vicinal *cis*-fluoroazides than in vicinal *cis*-fluoroamides due to a higher electronegativity of the azido with respect to the carboxamido function, is very evidently sensed in the fluorine resonances. The higher the population of pseudoaxially oriented fluorine atoms, the stronger its time-averaged chemical deshielding, the more downfield the ^{19}F resonance appears. The comparison of ^{19}F resonances between azide-amide pairs, in which all other atoms remain the same, reveal downfield shifts of $\Delta\delta_F$ (amide − azide) = 1.10 ppm for **10a**/**9a** ($CDCl_3$), 2.51 ppm for **13**/**14a** ($CDCl_3$), 2.72 ppm for **2** (CD_3OD)/**11a** (DMSO) and 3.40 ppm for **10b**/**9b** ($CDCl_3$). Recall that we observed the strongest downfield shift for the pseudoequatorial-to-pseudoaxial rearrangement of a fluorine (due to the formation of a stable hydrogen bridge) as we passed from $CDCl_3$ to DMSO of solutions of 2′-fluoro-3′-azides **11a** and **12**: $\Delta\delta_F$ = 8.6–8.7 ppm. In addition, useful information on equilibrium torsional angles can be extracted from F,H scalar coupling constants even without a corresponding Karplus–Conroy function, such as the ones that were very impressively and carefully worked out by J. Chattopadhyaya and co-workers.[28] In the compounds measured here we found vicinal F,H coupling constants ^3J(F,H) ≈19–29 Hz being up to three times higher than the highest vicinal H,H coupling constant measured for **10b**: ^3J(H,H) = 9.3 Hz (in other systems up to 15, max. 18 Hz). In other fluorinated compounds ^3J(F,H) limiting values of between 29.5 and 47 Hz for close to anti orientations have been found.[28] We are curious about a PSEUROT + $J_{H,F}$ analysis of our compounds, as well as that for arabinofuranose-configured 2′-deoxyfluoronucleoside derivatives such as Clofarabine.[32] Furthermore, ^{19}F resonances are far better sensors of diastereoisomeric forms than any other nucleus even though the fluorine atom might be at quite a distance from the symmetry breaking element, the phosphorous atom in compounds **13** and **14a** in our case. Last but not least, ^{19}F resonances sense the spacial proximity of hydrogen atoms, carbon atoms (through sometimes substantial long-range couplings) and also nitrogen atoms. The quadrupole of the latter nucleus (^{14}N) may find its imprint in the very evident broadening of the ^{19}F resonance making it difficult or impossible to determine the multiplicity in the fully coupled ^{19}F NMR signal, as can be readily seen for one of the two diastereoisomers of **13** and **14a** each and in all isomeric forms of the acyltriazenes **10c** and **10d**, but not so in **14b** (see Supporting Information). A close spacial proximity between F2′ and NNN3′ or between F3′ and NNN2′ giving rise to line broading of the ^{19}F resonance is not surprising. We were surprised, however, to see a line broading in only one of the diastereoisomers of **13** and **14a** and not the other, although the stereogenic centre is phosphorous six covalent bonds away from F2′. We were somewhat disappointed to see that the vicinal fluorine–carbon coupling constants in almost all of our compounds were too small to be useful, ^3J(F,C) < 1–2 Hz, despite the quite high geminal and direct F,C-coupling constants:

^2J(F,C) = 14–36 Hz, $|^1J$(F,C)$|$ = 184–196 Hz. One exception was South-puckered **11b** in $CDCl_3$ the rigid conformation of which not only produced a long range fluorine–proton coupling of ^4J(F3′α,H5′) = 3.0 Hz but also a vicinal F,C coupling of ^3J(F3′α,C5′) = 11.6 Hz (in agreement with compound **20** in the Supporting Information in ref. [23]), to the best of our knowledge, perhaps the highest measured vicinal fluorine–carbon coupling constant. According to the crystal structure coordinates ACADOS of 3′-*O*-acetyladenosine[29] this value should correspond to a time-averaged torsional angle of 142.5°. Apparently, South-puckered 3′-deoxyfluororibofuranose derivatives produce ^3J(F3′α,C5′) values between 9.1 and 11.6 Hz,[23] while some 2′-deoxyfluoroarabino nucleosides show

^3J(F2′β,C4′) = 6 and 10 Hz, and one reveals a close spacial proximity through a long-range ^4J(F2′β,C8β) = 7 Hz.[32] The only other substantial ^3J(F3′α,C5′) value that we could observe was 9.8 Hz in **10b**, the

only other high-South compound of which we measured a ^{13}C NMR spectrum (not shown).

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[59] The only comparable studies that we are aware of, that assumed the transient formation of a four-membered ring containing no other than second-row elements in their electronic ground state, are early publications on the mechanism of the spontaneous rearrangement of *N*-alkyl-*N*-nitrosoamides[60,61] and, later, *N*-alkyl-*N*-nitroamides,[61a,b] R-(O_{1-2}N)N-C(O)R′, into the corresponding *E*-configured diazoesters R-N=N$(O)_{0-1}$-O-C(O)R′ followed by the elimination of N_2 or N_2O, respectively, to give esters R-O-C(O)R′ and, concomitantly, acids HO-C(O)R′ and alkenes derived from R. Extensive kinetic studies on the reaction of *N*-alkyl-*N*-nitrosoamides identified a first-order rate-determining step, which could only be explained through the initial relatively slow formation of a four-membered R-*N*=*N*-*O*-*C*(O)R′ ring (*N*–*C* connected), described as a concerted intramolecular *N*,*O*-acyl shift, followed by another 1,3-shift of the R group from *N* to *O* to give the ester and dinitrogen or, alternatively, the elimination of R to give the acid, dinitrogen and the alkene. Today's credo seems to be (for some of us) that four-membered ring transition states, in order to be sufficiently stable, necessitate at least one at least third-row element in the ring, like in a Wittig or aza-Wittig reaction.

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