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Synthesis and Stereochemical Assignment of DNA Spore Photoproduct Analogues

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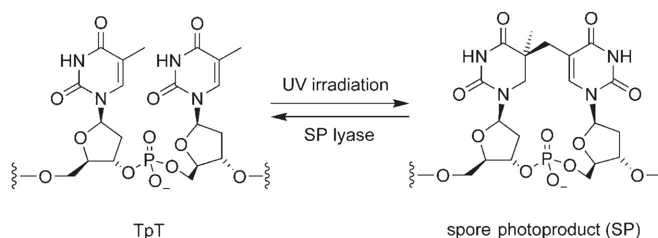
Abstract: Investigation of the DNA repair process performed by the spore photoproduct (SP) lyase repair enzyme is strongly hampered by the lack of defined substrates needed for detailed enzymatic studies. The problem is particularly severe because the repair enzyme belongs to the class of strongly oxygen-sensitive radical (*S*)-adenosylmethionine (SAM) enzymes, which are notoriously difficult to handle. We report the synthesis of the spore photoproduct analogues **1a** and **1b**, which have open backbones and are diastereoisomers. In order to solve the problem of stereochemical assignment, two further derivatives **2a** and **2b** with closed backbones were prepared. The key step of the synthesis of **2a/b** is a metathesis-based macrocyclization that strongly increases the conformational rigidity of the synthetic spore photoproduct derivatives. NOESY experiments of the cyclic isomers furnished a clear cross-peak pattern that allowed the unequivocal assignment of the stereochemistry. The results were transferred to the data for isomers **1a** and **1b**, which were subsequently used for enzymatic-repair studies. These studies were performed with the novel spore photoproduct lyase repair enzyme from *Geobacillus stearothermophilus*. The studies showed an accordance with a recent investigation performed by us with the spore photoproduct lyase from *Bacillus subtilis*,[1] in that only the *S* isomer **1a** is recognized and repaired. The ability to prepare a defined functioning substrate now paves the way for detailed enzymatic studies of the SP-lyase lesion recognition and repair process.

Keywords: cyclization · DNA damage · enzymes · spore photoproduct · structure elucidation

Introduction

Bacteria of the *Bacillus* and *Clostridium* species form metabolically dormant endospores in response to nutrient depletion.[2] Spores are resistant to toxic chemicals, heat, and desiccation, and can survive over extreme periods of time.[3] Among other factors, the unusual toroid packing of the genetic material inside the spore[4] is one factor believed to be responsible for the unusual 50-fold increased resistance of the spore DNA to 254 nm UV light.[5] Due to the unusual DNA packing, however, adjacent thymidines in the spore DNA react under exposure to UV light to give a unique DNA lesion, the spore photoproduct (SP) (Scheme 1).[6,7] This DNA lesion, which would inhibit spore germination, is efficiently repaired by the (*S*)-adenosylmethionine (SAM)-dependent iron sulfur DNA repair enzyme SP lyase in an early phase of spore germination.[8–14]

To date, the exact repair mechanism is unknown. Only a model study was carried out that suggests a plausible mechanism.[15] In addition, our knowledge about the exact struc-



Scheme 1. Formation and repair (SP lyase) of the spore photoproduct DNA lesion in UV-irradiated spores. TpT = thymidylyl–(3′–5′)–thymidine.

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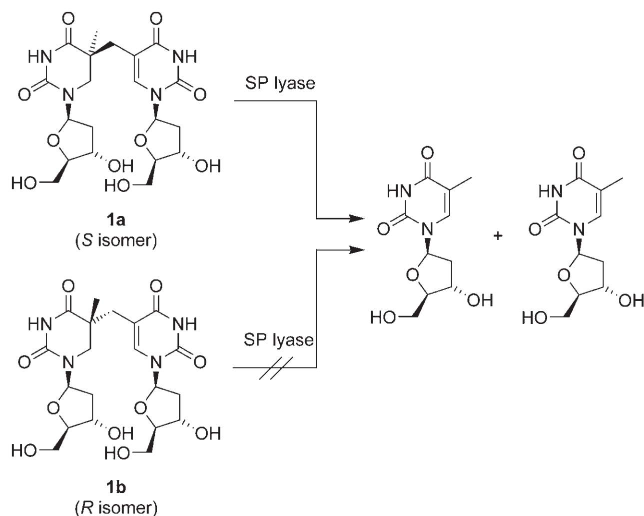
ture of the spore lesion itself and the reasons behind the formation of this photoproduct are only rudimentary. This has mainly to do with the fact that the lesion is currently not synthetically available due to the fact that the known synthesis of the dinucleotide spore photoproduct[16] has not delivered sufficient yields and therefore strongly limits enzymatic studies.[17] In principle, the SP lesion can be formed as the 5*S* or 5*R* stereoisomer in either an intra- or intermolecular fashion. It was revealed from a sophisticated high-performance liquid chromatography (HPLC)-MS/MS study[18] that the spore photoproduct is predominantly formed as an intrastrand lesion (99 %) and only to a minor extent as an interstrand cross-link. Regarding the stereochemistry, Begley et al.[16] postulated that the natural lesion might be formed as the 5*R* isomer. This postulate was based on the assumption that the DNA in spores is in a more A-like conformation. Within an A-type duplex, the structure would favor formation of the *R* isomer due to sterical constrains. A recent cryoelectronic investigation,[4] however, revealed that the spore DNA exists almost in the B conformation, questioning the sterical argument.

We recently described the use of SP-lesion analogues, which lack the central phosphodiester linkage **1a/b**, as defined substrates in a novel SP-lyase assay by using the SP-lyase enzyme from *Bacillus subtilis*. The enzymatic study showed clearly that only the *S* isomer **1a** is recognized and repaired by the repair enzyme (Scheme 2) indicating that

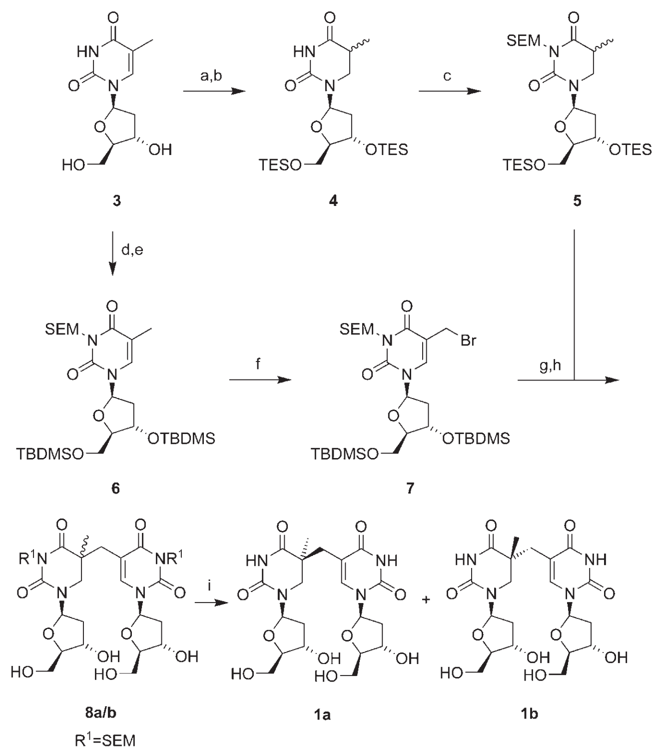


Scheme 2. Structure of compounds **1a** and **1b** that were tested in a repair assay with the spore photoproduct lyase.[1]

the natural lesion might be 5*S* and not 5*R* configured.[1] Herein we report the synthesis of lesion analogues **1a** and **1b** together with the derivatives with closed backbones, **2a/b**, which allowed the unequivocal assignment of the stereochemistry of both diastereomers by using 2D NMR spectroscopy (NOESY). Additionally, we report initial enzymatic-repair studies with the novel spore photoproduct lyase from the thermophilic organism *Geobacillus stearothermophilus*.

Results and Discussion

Synthesis of 1a/b and 2a/b: The synthesis of the substrates **1a/b** with open backbones was performed as depicted in Scheme 3.[1] The first steps were the hydrogenation of thymi-



Scheme 3. Synthesis of the substrates with open backbones (**1a/b**) starting with thymidine **3**. Reagents and conditions: a) Rh/Al_2O_3, H_2, MeOH/H_2O (1:1), RT; b) TESCl, imidazole, DMF, RT, 88 %; c) SEMCl, $i$$Pr_2$NEt, CH_2Cl_2, RT, 84 %; d) TBDMSCl, imidazole, DMF, RT, 95 %; e) SEMCl, $i$$Pr_2$NEt, CH_2Cl_2, RT, 72 %; f) NBS, DBPO, CCl_4, 70 °C, 60 %; g) LDA (1.5 equiv), **5**, THF, −78→0 °C; h) TBAF, THF, RT; rp-HPLC separation, **8a**: 15 %, **8b**: 11 %; i) $SnCl_4$, DCM, 0 °C, 55 % (for **1b**: 75 %).

dine **3** under atmospheric pressure using Rh/Al_2O_3 as a catalyst, followed by the protection of the OH groups using TESCl (TES = triethylsilyl) to give compound **4**, and further protection of the NH group using SEMCl (SEM = trimethylsilylethoxymethyl) to give the desired dihydrothymidine building block **5**. A second batch of thymidine **3** was treated with TBDMSCl (TBDM = *tert*-butyldimethylsilyl) and SEMCl to give the TBDMS- and SEM-protected compound **6**. The following bromination furnished the allylbromide **7** as the second building block needed for the critical coupling reaction. For the coupling of **5** with **7**, we first deprotonated compound **5** with LDA and allowed the Li enolate to react with the allylbromide **7**. Subsequent deprotection of the OH groups using tetrabutylammonium fluoride (TBAF) gave the SEM-protected diastereomers **8a** and **8b** as a 1:1 mixture. Compounds **8a** and **8b** were finally separated by reversed-phase (rp) HPLC (120 Å, 3 μm, C8). Cleavage of the SEM protecting groups was possible using $SnCl_4$, which fur-

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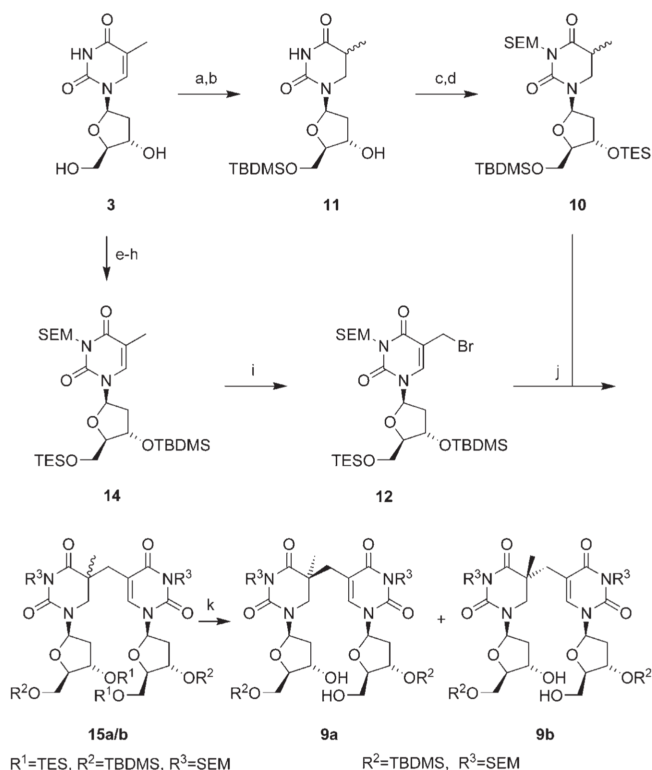
nished the SP-lesion monomers **1a** and **1b** in an overall yield of 0.7% for each diastereomer.

Quantitative NOESY experiments of **1a** and **1b** to assign the stereoconfiguration at C5 of the dihydrothymidine unit gave no significant results due to the high flexibility of the molecules. Particularly problematic is the ability of the molecules to freely rotate around the glycosidic bond.

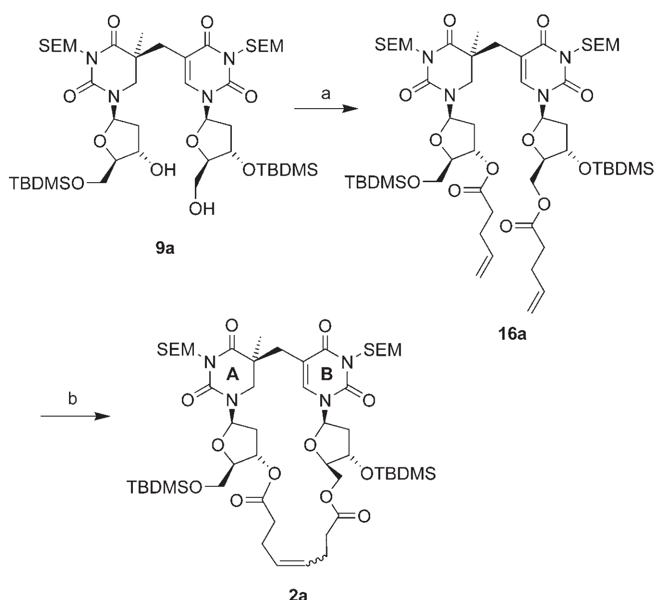
In order to increase the rigidity of the SP isomers we developed a strategy to perform a macrocyclization (ring-closing metathesis, RCM) with the TBDMS-protected isomers **9a/b**, which were synthesized as shown in Scheme 4. The dihydrothymidine building block **10** was synthesized in four steps analogously to building block **5**. Thymidine **3** was first hydrogenated and subsequently protected in the 5′-position with TBDMSCl to give the 5′-TBDMS-dihydrothymidine **11**. Protection of the OH(3′) group was performed with TESCl. Final protection of the ring imide with SEMCl gave the dihydrothymidine building block **10**.



Scheme 4. Synthesis of the spore photoproduct analogues **9a/b** starting with thymidine **3**. Reagents and conditions: a) Rh/Al_2O_3, H_2, MeOH/H_2O (1:1), RT; b) TBDMSCl, imidazole, DMF, RT, 82%; c) TESCl, imidazole, DMF, RT, 89% d) SEMCl, iPr_2NEt, CH_2Cl_2, RT, 86%; e) TBDMSCl, imidazole, DMF, RT, 95%; f) TFA/H_2O (10:1), CH_2Cl_2, 0°C, 81%; g) TESCl, imidazole, DMF, RT, 91%; h) SEMCl, iPr_2NEt, CH_2Cl_2, RT, 64%; i) NBS, DBPO, CCl_4, 70°C, 20%; j) LDA (1.5 equiv), **10**, THF, −78→0°C; k) HF•pyr (4%), MeCN, −30°C, 34%.

The allylbromide building block **12** was synthesized in five steps also starting from thymidine **3**. First, both OH groups were protected with TBDMS. In a second step, the 5′-protecting group was selectively cleaved with TFA/H_2O to furnish the 3′-TBDMS-thymidine **13**. Following protection with TESCl and SEMCl we obtained the completely protected thymidine **14** in an overall yield of 41%. Subsequent bromination with NBS furnished the allylbromide **12**. Deprotonation of the dihydrothymidine compound **10** with LDA and coupling of the enolate with the allylbromide **12** afforded the methylene-linked bis-thymidine compound again in the form of two diastereoisomers (**15a+15b**). Deprotection with HF•pyridine gave the desired protected SP analogues **9a/b**. The diastereomers **9a** und **9b** were separated by using normal-phase (np) HPLC (100 Å, 5 μm) with an *n*-heptane/ethyl acetate gradient.

For the cyclization of the separated isomers **9a** and **9b** (Scheme 5), both compounds were first reacted with 4-pentenoylchloride to give the diesters **16a** and **16b**. The macrocyclization was finally achieved through an RCM reaction[19] with the Grubbs II catalyst.[20,21] This reaction furnished the 24-membered rings **2a** and **2b** as *E*/*Z* mixtures (**2a**, 95:5; **2b**, 85:15; Scheme 5).[22] The *E*/*Z* isomers were separated by using np-HPLC (100 Å, 5 μm) with an *n*-hexane/ethyl acetate gradient. Only the major isomers were used for further investigation. The exact geometry of the double bond of the major isomers **2a** and **2b** could not be determined due to the complex coupling pattern and the lack of resolution of the double-bond proton signals in the NMR spectra (see the Supporting Information for the ^{1}H NMR spectra).



Scheme 5. Synthesis of the cyclic SP isomer **2a** (the synthesis of **2b** was performed under the same conditions). Reagents and conditions: a) 4-pentenoylchloride, iPr_2NEt, CH_2Cl_2, RT, 94% (for **16b**: 80%); b) Grubbs II catalyst, CH_2Cl_2, 40°C, 71% (for **2b**: 82%). The HPLC-purified cyclic isomers **2a** and **2b** were analyzed by 2D NMR (NOESY) to assign the stereochemistry at C5 of the dihydrothymidine unit.

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NMR investigations of 2a and 2b: For the 2D NMR analyses, all ^{1}H and ^{13}C NMR signals of **2a** and **2b** were assigned by using ^{1}H,^{1}H COSY, ^{1}H,^{13}C COSY (HMQC), and HMBC experiments. NOESY experiments were performed for the stereochemical assignment of the stereo center at C5 of the dihydrothymidine unit.

In the case of isomer **2a**, the proton in the 6-position of the pyrimidine ring (B) shows NOE contacts to H(3′), H(5′), and H(2′) above the sugar plane. Therefore, H(6B) is positioned above the sugar ring and the base is in the *anti* conformation (Figure 1D). The CH_2 group in the 6-position of the dihydrothymidine unit (A) shows NOE contacts to the methyl group that are almost identical for both protons (Figure 1A). This shows that the methyl group is positioned between the two H atoms (H_a and H_b). The proton of this CH_2 group with a signal at $\delta = 3.33$ ppm (H_a) features a strong cross peak to the proton in the 2′-position ($\delta = 2.15$ ppm (H_c), Figure 1B) above the sugar ring (Figure 2A). In addition, proton H_a shows a strong NOE contact to the proton, with a signal at $\delta = 2.68$ ppm (H_e), of the CH_2 bridge together with a weaker contact to the proton with a signal at $\delta =$ 2.28 ppm (H_f, Figure 1C). The proton H_e features a strong cross peak to the methyl group (Figure 2B) and the proton H_f a strong NOE contact to the CH(6B) proton (Figure 1D).

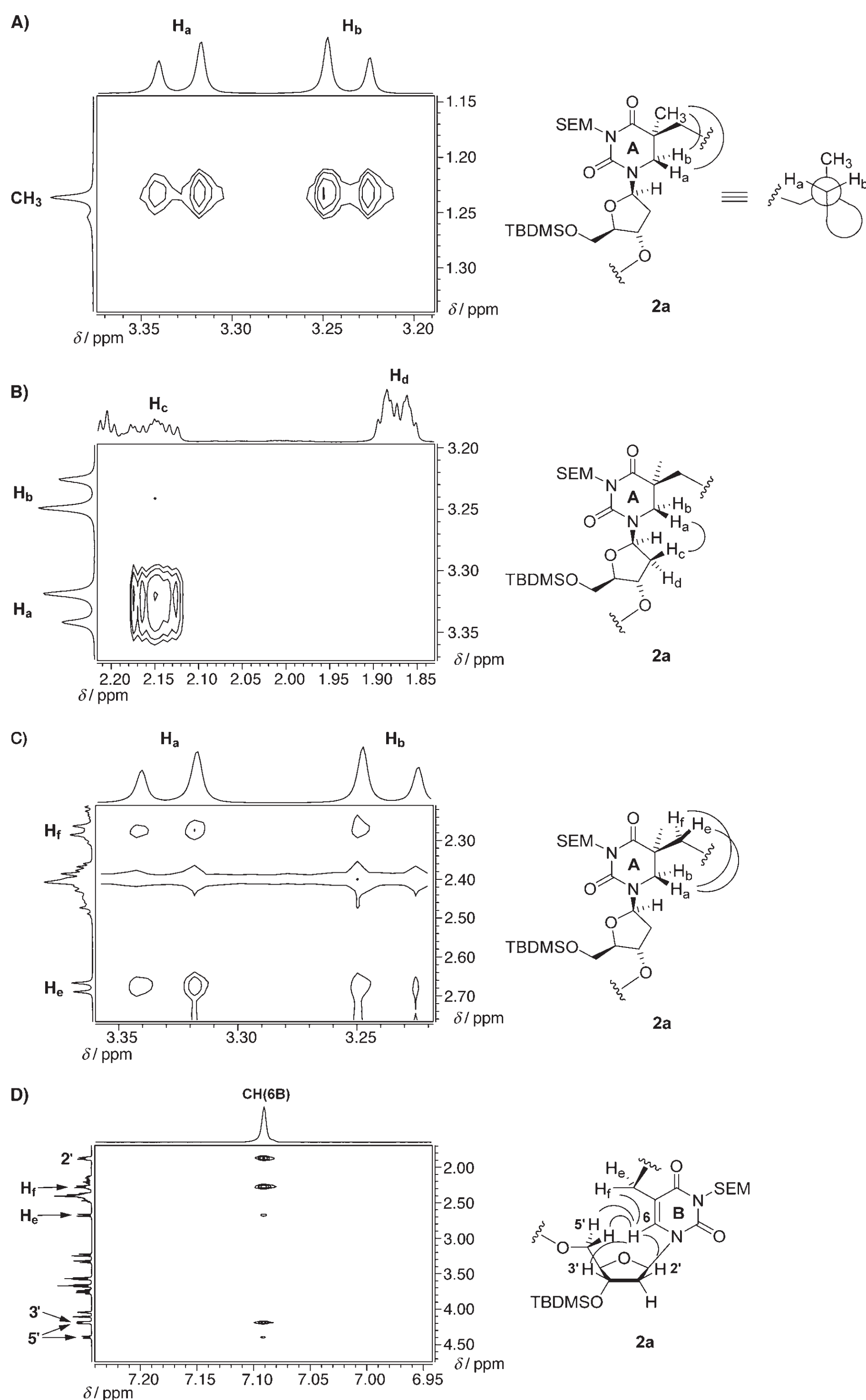

Figure 1. Depiction of the first set of essential NOESY cross peaks in $CDCl_3$ (600 MHz) for the assignment of the stereoconfiguration of **2a**.

All together, this cross-peak pattern can only be explained when the CH_2 bridge is pointing to the front and the methyl group to the back. This, however, is only possible when isomer **2a** is *S* configured (Figure 3). This assignment is supported by additional cross peaks between the CH_2 group (6A) and H(1′), which indicate that the H atoms of the CH_2 group are not located above the sugar ring (Figure 2C) excluding other conformations that would be in agreement with the observed cross-peak pattern. Hence, we can conclude that isomer **2a** is the *S*-configured compound.

For isomer **2b**, the pyrimidine ring (B) is also in the *anti* conformation. The H(6) proton with signal at $\delta = 7.41$ ppm possesses NOE contacts to the sugar protons above the ring

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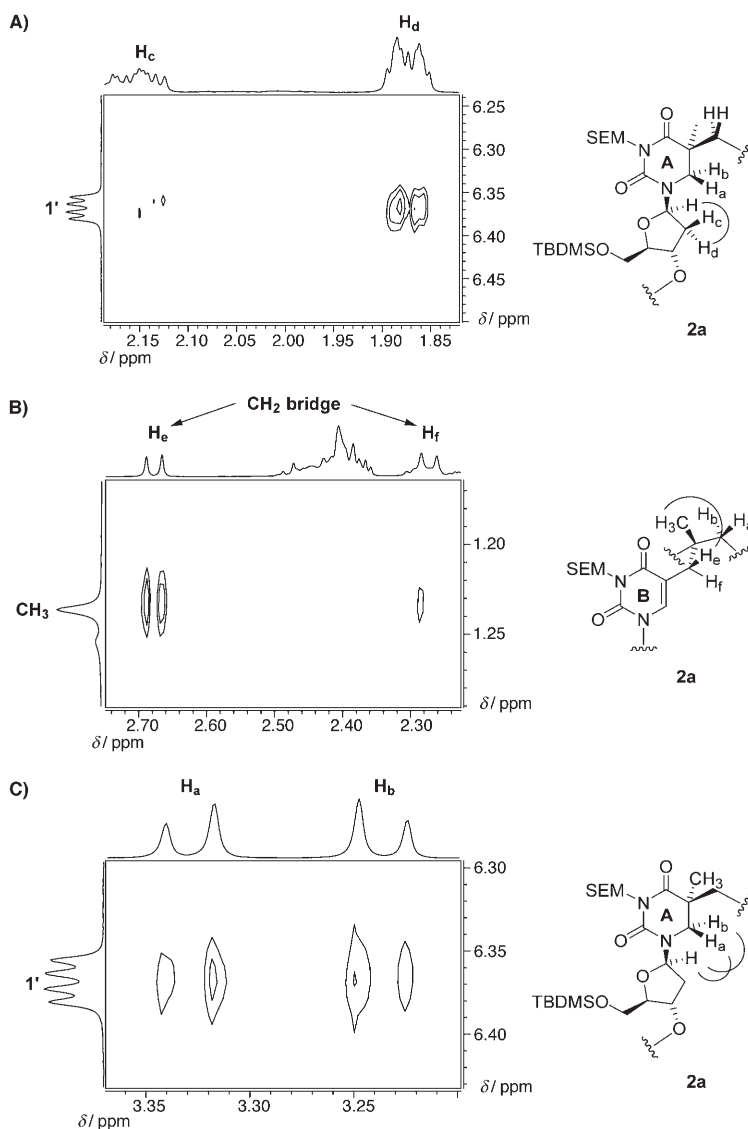

Figure 2. Depiction of the second set of essential NOESY cross peaks in $CDCl_3$ (600 MHz) for the assignment of the stereoconfiguration of **2a**.

tacts to the proton in the 2′-position (H_c) with almost the same intensity (Figure 4B). So proton H_c is located in the middle between the protons of the CH_2 group. Additional cross peaks to other sugar protons are not present. Contacts to the CH_2 bridge between the bases are observed only for proton H_b ($\delta = 3.67$ ppm). The cross peak to the H atom with a signal at $\delta = 2.75$ ppm (H_e) is slightly larger relative to the cross peak to the H atom with a signal at $\delta = 2.68$ ppm (H_f, Figure 4B). The distance from the CH_2 group (6A) to the methyl group is almost identical for both protons (Figure 4C). In addition, NOE contacts between the CH_2 bridge and the methyl group were observed; a strong one to the signal at $\delta = 2.68$ ppm (H_f) and a weaker one to the signal at $\delta = 2.75$ ppm (H_e, Figure 4C). The CH(6B) proton shows a strong cross peak to the CH_2 bridge proton with a signal at $\delta = 2.75$ ppm (H_e, Figure 4A), which is the same proton that the CH_2(6A) protons showed a cross peak to (H_b, Figure 4B), and a weaker cross peak to the proton with a signal at $\delta = 2.68$ ppm (H_f), which shows a strong signal to the methyl group (Figure 4C).

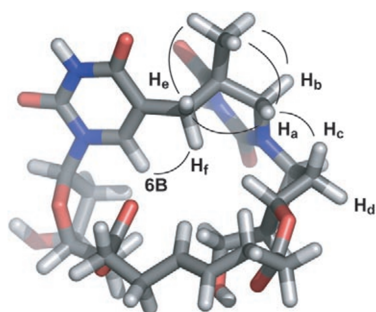

Figure 3. Three-dimensional depiction of **2a** in the *S* configuration showing the observed strong NOE contacts. The SEM and TBDMS protecting groups were exchanged for H atoms for clarity.

plane (H(5′), H(2′)/H_c, and H(3′); Figure 4A). The two protons of the CH_2 group ($\delta = 3.14$ (H_a) and 3.67 ppm (H_b)) at the 6-position of the dihydrothymidine unit show NOE con-

In principle, the observed NOESY signal pattern for **2b** could be explained by both the 5*S* and 5*R* stereochemistry. But, in the case of the *S* configuration, large sterical interactions between the 5′-TBDMS group and the backbone as well as the bases would have to be accepted, which is unlikely (Figure 5, right). In the case of the *R* configuration, sterical repulsion is negligible (Figure 5, left). Consequently, we assign the *R* configuration to isomer **2b**.

In order to correlate the results of the stereochemical assignment of compounds **2a/b** to the fully deprotected SP isomers **1a/b**, small amounts of **9a** and **9b** were deprotected by using TBAF to generate the SEM-protected diastereomers **8a** and **8b** as depicted in Scheme 6. These isomers were also isolated during the synthesis of **1a/b** and separated by using rp-HPLC (see above and ref. [1]).

A comparison of the HPL chromatograms from **8a** and **8b** derived either from **9a** and **9b** or from the synthesis of

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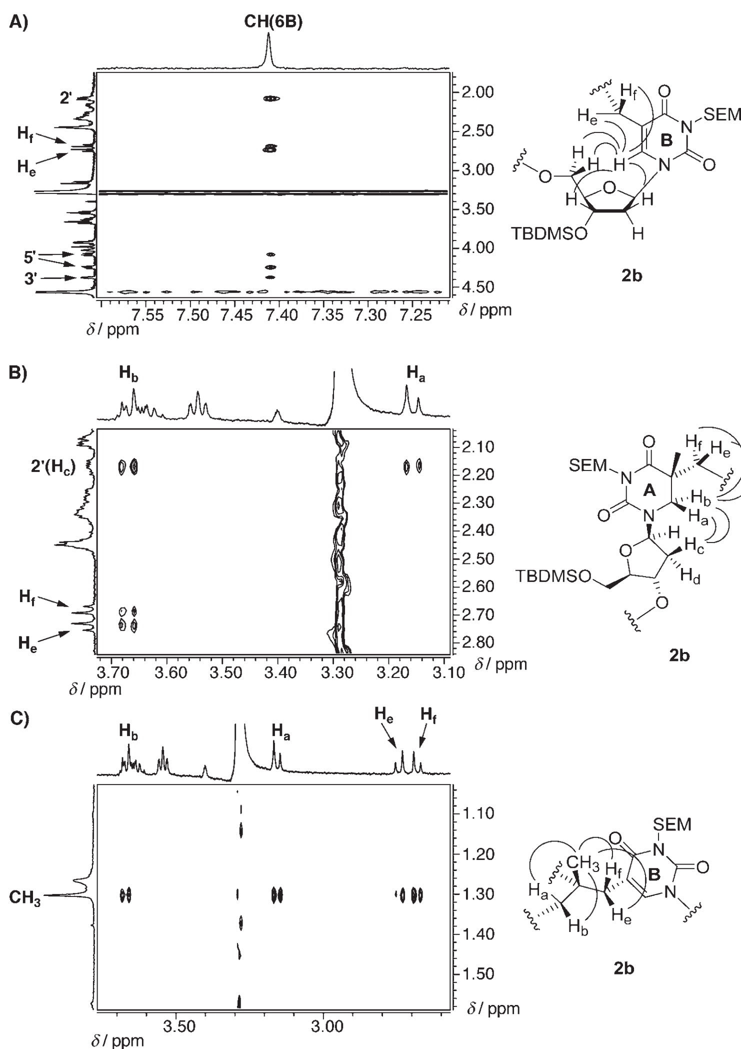

Figure 4. Depiction of the set of essential NOESY cross peaks in MeOH (600 MHz) for the assignment of the stereoconfiguration of **2b**.

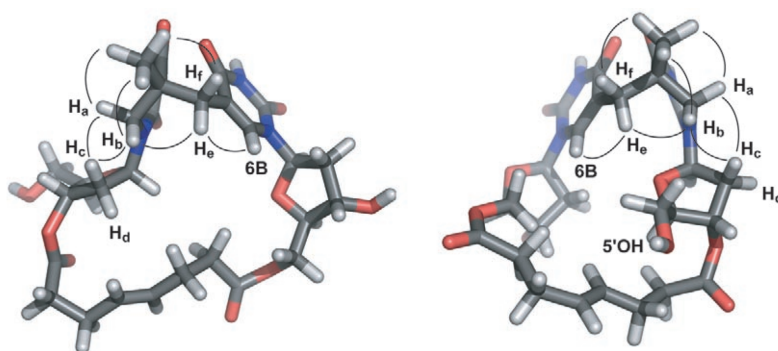

Figure 5. Three-dimensional depiction of **2b** in the *R* (left) and *S* (right) configuration showing the observed strong NOE contacts. The SEM and TBDMS protecting groups were exchanged for H atoms for clarity.

1a and **1b** shows that the order of appearance stays the same (see the Supporting Information). Therefore, **1a** must be the *S* isomer and **1b** the *R* isomer of the fully deprotected SP isomers. To ensure the identity of the deprotected isomers **8a** and **8b**, samples corresponding to the HPLC peaks were collected and the mass was determined with MALDI-TOF analysis (see the Supporting Information).

Enzymatic studies: With the unequivocally assigned diastereomers **1a** and **1b** as defined substrates in hand, we performed enzymatic studies with the purified and reconstituted spore photoproduct lyase from *G. stearothermophilus* (splG).[8,11,23–25] We performed enzymatic assays under different conditions and analyzed them by using rp-HPLC following a previously described method.[1] The enzyme contains a reduced $[4Fe+4S]^+$ cluster that is believed to transfer an electron to the SAM cofactor, resulting in the formation of methionine (Met) and the highly reactive 5′-deoxyadenosine radical (Scheme 7). This unstable radical is highly reactive and forms 5′-deoxyadenosine (5′AdoH), which can be easily detected at $\lambda = 260$ nm by using HPLC.

This was tested in the first assay solution containing only SAM and splG. As shown in Figure 6A, we could observe the formation of 5′AdoH. The production of 5′AdoH by the holo-splG is 1.2 molecules of 5′-deoxyadenosine per molecule of enzyme. In a second batch we added the *R* isomer **1b** to the assay solution and observed only the peaks for 5′AdoH and compound **1b** (Figure 6B). In a third batch we added the *S* isomer **1a**. Only here we observed the formation of a new peak at 12.9 min, which corresponds to the desired repair product, thymidine (Figure 6C). The thymidine formation is time dependent and the calculated reaction yield for the splG-driven repair of the *S*-isomer substrate is 0.2 mol product formation per 1 mol splG per hour. In a control experiment containing only compound **1a** and SAM and no splG

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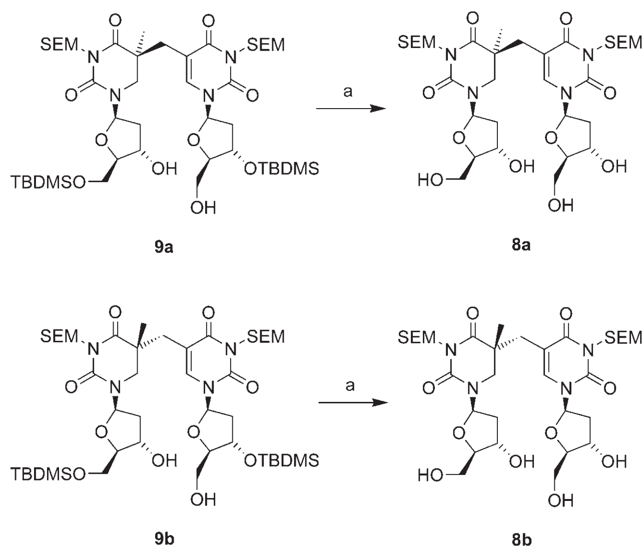
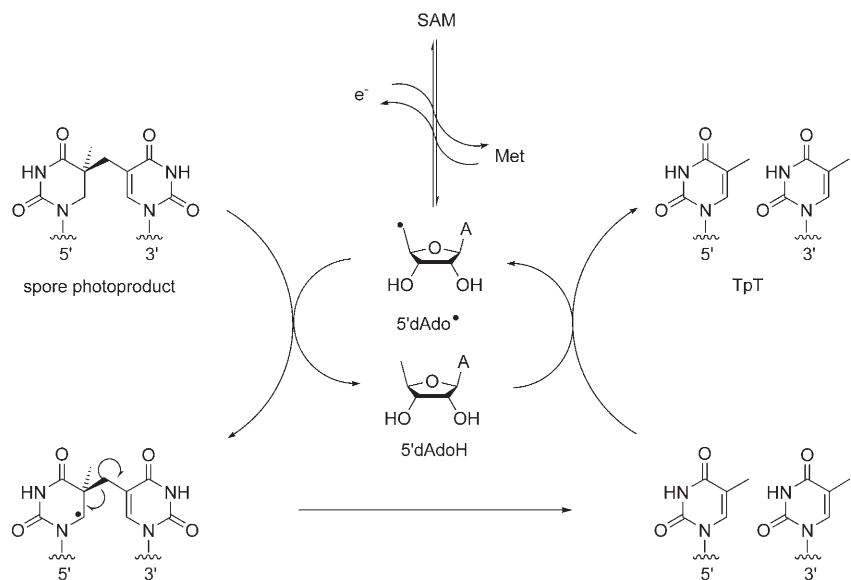


Scheme 6. Deprotection of isomers **9a** and **9b**. Reagents and conditions: a) TBAF, THF, RT.



Scheme 7. Postulated repair mechanism of the spore photoproduct lyase.[8,15]

enzyme, we neither observed the formation of 5′AdoH nor the formation of thymidine (Figure 6D) showing that their formation is strictly enzyme dependent.

The formation of 5′AdoH and thymidine was confirmed by coinjection (HPLC) and mass spectrometry. In these enzymatic studies we observed, as in the case of the recently published study with the spore photoproduct lyase from *Bacillus subtilis*,[1] that only the *S* isomer **1a** is accepted as a substrate and repaired by the enzyme. These investigations, performed with the SPL enzyme from a different organism, therefore support the idea that the spore repair enzymes can only repair the 5*S* isomers of the spore photoproduct.

Conclusion

In summary, we were able to synthesize and separate the diastereomers with open backbones, **1a** and **1b**, which function as substrates for the DNA repair enzyme SP lyase from spores. The stereochemical assignment of the synthetic SP isomers **1a** and **1b** was possible by using the cyclic SP isomers **2a** and **2b**. The key step of the synthesis of **2a/b** was a macrocyclization to increase the rigidity of the spore photoproduct derivatives. In NOESY experiments of the cyclic isomers we obtained cross-peak patterns that allowed the assignment of the stereochemistry for both diastereomers **2a** and **2b**. We were able to correlate these results to isomers **1a** and **1b** to assign the stereochemistry of the lesion analogues. Additionally, enzymatic DNA repair studies using **1a** and **1b** as defined substrates and the SP lyase from *G. stearothermophilus* were performed. These studies showed, as in the case of our recently published repair study with the spore photoproduct lyase from *B. subtilis*,[1] that only the *S* isomer **1a** is recognized and repaired by splG. These synthetic stereochemically defined and pure spore photoproduct derivates pave the way for detailed enzymatic studies of the SP-lyase lesion recognition and repair process.

Experimental Section

General information: All solvents were of the quality puriss. p.a., or purum. Purum solvents were distilled prior to use. The commercially available reagents were used as received without further purification. Analytical thin-layer chromatography (TLC) was carried out using aluminum-based plates (Kieselgel 60 F_{254}) from Merck. Plates were visualized under UV light ($\lambda = 254$ nm) or by staining them with anisaldeyhde solution. Flash chromatography was carried out by using Merck Kieselgel 60 (0.040–0.063 mm) with N_2 overpressure. Samples were applied as saturated solutions in an appropriate solvent. Melting points are uncorrected. ^{1}H NMR spectra were recorded on Bruker DRX 200 (200 MHz), AMX 300 (300 MHz), ARX 300 (300 MHz), AMX 400 (400 MHz), AMX 500 (500 MHz), AMX 600 (600 MHz), Varian Oxford 200 (200 MHz), and Varian XL 400 (400 MHz) spectrometers. The chemical shifts were referenced to DMSO ($\delta = 2.50$ ppm) in [D$_6$]DMSO, CH_3OH ($\delta = 3.31$ ppm) in CD_3OD, and $CHCl_3$ ($\delta = 7.26$ ppm) in $CDCl_3$. ^{13}C NMR spectra were recorded on Bruker ARX 200 (50 MHz), AMX 300 (75 MHz), ARX 300 (75 MHz), AMX 500 (125 MHz), AMX 600 (150 MHz), and Varian XL 400 (100 MHz) spectrometers. The chemical shifts were referenced to DMSO ($\delta = 39.43$ ppm) in [D$_6$]DMSO, CH_3OH ($\delta = 49.05$ ppm) in CD_3OD, and $CHCl_3$ ($\delta = 77.00$ ppm) in $CDCl_3$. Standard pulse sequences were employed for ^{1}H,^{1}H and ^{1}H,^{13}C NMR correlation studies. IR spectra of solids were recorded in KBr, and of liquids as thin films. IR spectra were measured with Bruker IFS 88 and Perkin–Elmer FTIR spectrum 100 in-

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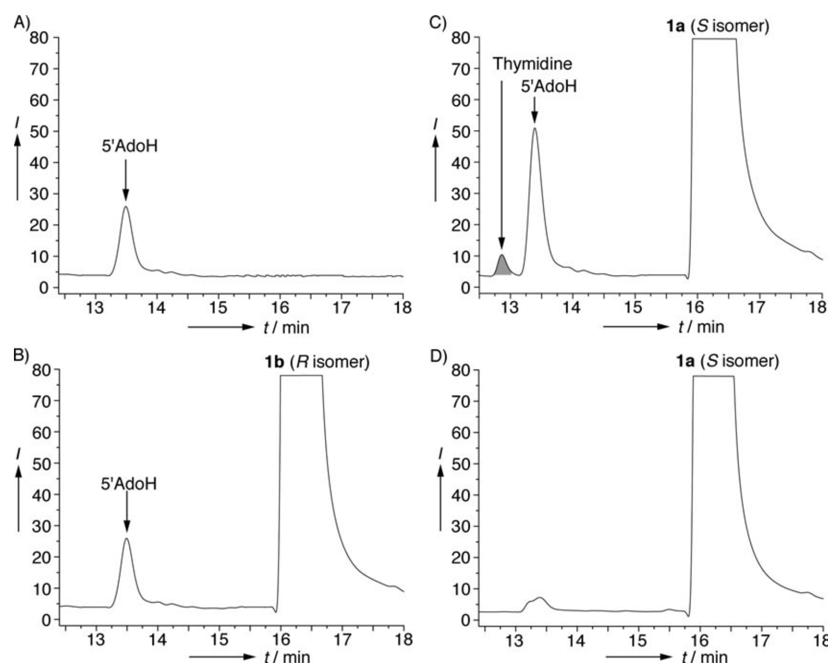

Figure 6. HPL chromatograms of enzymatic studies. The enzyme reaction and rp-HPLC analyses were performed as described in the Experimental Section. A) HPLC analysis of the assay containing 5 nmol splG and SAM. B) HPLC analysis of the assay containing 5 nmol splG, SAM, and the (*R*)-**1b**. C) HPLC analysis of the assay containing 5 nmol splG, SAM, and (*S*)-**1a**. Only in this case an additional peak was observed, which was identified as the desired repair product thymidine. D) HPLC analysis of the negative control containing SAM and (*S*)-**1a** but no splG. In all four chromatograms the *y* axis shows the intensity of the absorbance at $\lambda = 260$ nm.

struments. Mass spectra and high-resolution mass spectra were measured on Finnegan TSQ 7000, Finnegan MAT 95S, Finnegan MAT 95Q, Finnegan MAT 90 (FAB), Finnegan LTQ-FT, PE Sciex Q-Star Pulsar i, and Bruker Autoflex II (MALDI-TOF) instruments. Analytical HPLC was performed with Merck–Hitachi systems equipped either with L-7400 UV and L-7480 fluorescence detectors or with L7420 UV/Vis and L-7455 DAD detectors. Preparative HPLC was performed with a Merck–Hitachi system equipped with a L-7480 UV detector. Analytical separations were performed with Macherey–Nagel Nucleosil 120-3 C8, Nucleosil 100-5, and Nucleosil 120-3 columns. Preparative separations were performed with Macherey–Nagel Nucleosil 120-3 C8 and Nucleodur 100-5 columns.

NMR measurements: All NMR spectra for the stereochemical analysis were recorded at 298 K on a 600 MHz Bruker DMX spectrometer (Bruker, Karlsruhe, Germany) equipped with a quadrupole resonance probe head with actively shielded *x*, *y*, and *z* gradients. The experiments were carried out on a 18 mM sample of **2a** (9 mg dissolved in 500 µL $CDCl_3$) and a 16 mM sample of **2b** (8 mg dissolved in 500 µL CD_3OD). The spectra were processed by using XWINNMR (Bruker) and analyzed with either XWINNMR or SPARKY software.[26] Resonance assignments were obtained from standard ^{1}H TOCSY,[27,28] ^{1}H COSY,[29,30] ^{13}C,^{1}H HSQC,[31–33] and ^{13}C,^{1}H HMBC[34] spectra. Stereospecific assignments of the prochiral methylene groups were derived from NOESY spectra, which were acquired with a mixing time of 150 ms and suppression of zero quantum artifacts.[35,36] The spectra were recorded with 32 scans per increment. The spectral width was 6009 Hz in both the direct and the indirect dimension, sampled with 2048 and 512 complex points, respectively. All dimensions were apodized with a π/2-shifted square sine-bell function zero filling to provide a processed spectrum of 4096×1024 complex points.

3′,5′-*O*-Di(triethylsilyl)-5,6-dihydrothymidine (4):[16,37] Thymidine **3** (2.00 g, 8.26 mmol) was dissolved in MeOH/water (50 mL, 1:1) and Rh/Al_2O_3 (100 mg, 5% Rh) was added. The suspension was stirred under an H_2 atmosphere at RT for 3 d. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure. The crude product was azeotropically dried three times with pyridine and dissolved in anhydrous DMF (20 mL). Imidazole (3.00 g, 44.1 mmol) and TESCl (4.16 mL, 24.8 mmol) were added and the reaction mixture was stirred overnight at RT. The reaction mixture was diluted with $CHCl_3$ (30 mL), washed with saturated aqueous sodium bicarbonate (3×40 mL), dried ($MgSO_4$), and the solvent was removed in vacuo. Purification by using flash chromatography (silica gel, pentane/ethyl acetate 7:3) provided **4** as a white solid (3.45 g, 88%).

R_f = 0.35 (pentane/ethyl acetate 7:3); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.52–0.62 (m, 12H; 2×Si(CH_2CH$_3$)$_3$), 0.87–0.96 (m, 18H; 2×Si(CH$_2$$CH_3$)$_3$), 1.05 (d, *J* = 6.6 Hz, 3H; C(5)CH_3), 1.73–1.82 (m, 1H; CH_2(2′)), 2.11–2.22 (m, 1H; CH_2(2′)), 2.55–2.64 (m, 1H; *CH*(5)), 2.91–2.99 (m, 1H; CH_2(6)), 3.35–3.40 (m, 1H; CH_2(6)), 3.55–3.68 (m, 3H; *CH*(4′), CH_2(5′)), 4.22–4.33 (m, 1H; *CH*(3′)), 6.11 (t, *J* = 6.6 Hz, 1H; *CH*(1′)), 10.23 ppm (s, 1H; N*H*); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 4.0 (Si(CH_2CH$_3$)$_3$), 4.3 (Si(CH_2CH$_3$)$_3$), 6.8 (2×Si(CH$_2$$CH_3$)$_3$), 12.7 (C(5)$CH_3$), 34.8 (*C*H(5)), 36.5 (*C*H$_2$(2′)), 41.5 (*C*H$_2$(6)), 62.7 (*C*H$_2$(5′)), 72.1 (*C*H(3′)), 83.1 (*C*H(1′)), 85.6 (*C*H(4′)), 153.2 (*C*O), 173.3 ppm (*C*O); MS (FAB$^+$): *m*/*z* (%): 495 (20) [*M*+Na]$^+$, 443 (13), 413 (6), 311 (10), 213 (18), 145 (40), 115 (93), 87 (100), 59 (36).

***N*^{3}-Trimethylsilylethoxymethyl-3′,5′-*O*-di(triethylsilyl)-5,6-dihydrothymidine (5)**:[16] A solution containing **4** (3.64 g, 7.70 mmol), *i*Pr$_2$NEt (5.27 mL, 30.8 mmol), and SEMCl (2.04 mL, 11.6 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred for 4 d. After 24 h additional *i*Pr$_2$NEt (5.27 mL, 30.8 mmol) and SEMCl (2.04 mL, 11.6 mmol) were added. The reaction mixture was diluted with $CHCl_3$ (30 mL), washed with saturated aqueous sodium bicarbonate (3×50 mL), dried ($MgSO_4$) and the solvent was removed in vacuo. Flash chromatography (silica gel, pentane/ethyl acetate 10:1) gave **5** as a colorless oil (3.89 g, 84%).

R_f = 0.43 (pentane/ethyl acetate 9:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = −0.04 (s, 9H; Si(CH_3)$_3$), 0.54–0.63 (m, 12H; 2×Si(CH_2CH$_3$)$_3$), 0.81 (t, *J* = 7.8 Hz, 2H; SiCH_2CH$_2$O), 0.90–0.96 (m, 18H; 2×Si(CH$_2$$CH_3$)$_3$), 1.11 (d, *J* = 7.0 Hz, 3H; C(5)CH_3), 1.81–1.88 (m, 1H; CH_{2a}(2′)), 2.15–2.24 (m, 1H; CH_{2b}(2′)), 2.72–2.79 (m, 1H; *CH*(5)), 2.99 (dd, *J* = 12.3, *J* = 10.3 Hz, 1H; CH_{2a}(6)), 3.41 (dd, *J* = 12.9, *J* = 5.6 Hz, 1H; CH_{2b}(6)), 3.50 (t, *J* = 8.0 Hz, 2H; SiCH$_2$$CH_2$O), 3.60–3.62 (m, 2H; CH_2(5′)), 3.66–3.68 (m, 1H; *CH*(4′)), 4.30–4.32 (m, 1H; *CH*(3′)), 5.05 (s, 2H; OCH_2N), 6.17 ppm (t, *J* = 7.3 Hz, 1H; *CH*(1′)); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = −1.2 (Si(CH_3)$_3$), 4.0 (Si(CH_2CH$_3$)$_3$), 4.3 (Si(CH_2CH$_3$)$_3$), 6.7 (2×Si(CH$_2$$CH_3$)$_3$), 13.1 (C(5)$CH_3$), 17.6 (OCH$_2$$CH_2$Si), 35.1 (*C*H(5)), 36.7 (*C*H$_2$(2′)), 40.4 (*C*H$_2$(6)), 62.6 (*C*H$_2$(5′)), 65.8 (OCH_2CH$_2$Si), 69.3 (NCH_2O), 72.0 (*C*H(3′)), 84.0 (*C*H(1′)), 85.8 (*C*H(4′)), 152.9 (*C*O), 172.6 ppm (*C*O); MS (FAB$^+$): *m*/*z* (%): 625 (8) [*M*+Na$^+$], 603 (3) [*M*+H$^+$], 573 (5), 427 (5), 213 (13), 145 (48), 116 (100), 87 (71), 73 (53), 59 (23), 44 (11).

3′,5′-*O*-Di(*tert*-butyldimethylsilyl)thymidine (17):[38,39] Thymidine **3** (2.00 g, 8.26 mmol) was dissolved in anhydrous DMF. Imidazole (3.38 g, 49.6 mmol) and TBDMSCl (3.74 g, 24.8 mmol) were added and the solution was stirred overnight at RT. The reaction mixture was diluted with $CHCl_3$ (200 mL), washed with water (3×200 mL), dried ($MgSO_4$), and the solvent was removed in vacuo. Purification by using flash chromatog-

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raphy (silica gel, pentane/ethyl acetate 1:1) provided **17** as a white foam (3.70 g, 95 %).

R_f=0.30 (CHCl$_3$/MeOH 20:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=0.06 (s, 6H; Si(CH$_3$)$_2$), 0.07 (s, 6H; Si(CH$_3$)$_2$), 0.86 (s, 9H; C(CH$_3$)$_3$), 0.87 (s, 9H; C(CH$_3$)$_3$), 1.76 (s, 3H; C(5)CH$_3$), 2.00–2.09 (m, 1H; CH$_{2a}$(2′)), 2.12–2.22 (m, 1H; CH$_{2b}$(2′)), 3.67–3.74 (m, 2H; CH$_2$(5′)), 3.75–3.79 (m, 1H; CH(4′)), 4.32–4.38 (m, 1H; CH(3′)), 6.14 (t, J=6.3 Hz, 1H; CH(1′)), 7.41 (s, 1H; CH(6)), 11.33 ppm (s, 1H; NH); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=−5.3 (SiCH$_3$), −5.3 (SiCH$_3$), −4.8 (SiCH$_3$), −4.6 (SiCH$_3$), 12.4 (C(5)CH$_3$), 17.9 (C(CH$_3$)$_3$), 18.2 (C(CH$_3$)$_3$), 25.8 (C(CH$_3$)$_3$), 25.9 (C(CH$_3$)$_3$), 39.3 (CH$_2$(2′)), 62.9 (CH$_2$(5′)), 72.2 (CH(3′)), 83.8 (CH(1′)), 86.8 (CH(4′)), 109.7 (C(5)), 135.7 (CH(6)), 150.6 (CO), 163.8 ppm (CO); MS (FAB$^+$): m/z (%): 493 (35) [M+Na$^+$], 471 (17) [M+H$^+$], 281 (10), 213 (13), 145 (46), 127 (15), 115 (16), 89 (40), 73 (100).

N^3-Trimethylsilylethoxymethyl-3′,5′-*O*-di(*tert*-butydimethylsilyl)thymidine (6):[16] SEM protection was carried out as described above for **5**. Product **6** was isolated as a colorless oil (3.34 g, 72 %).

R_f=0.33 (pentane/ethyl acetate 9:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=−0.04 (s, 9H; Si(CH$_3$)$_3$), 0.08 (s, 6H; Si(CH$_3$)$_2$), 0.09 (s, 6H; Si(CH$_3$)$_2$), 0.81–0.85 (m, 2H; SiCH$_2$CH$_2$O), 0.88 (s, 9H; C(CH$_3$)$_3$), 0.89 (s, 9H; C(CH$_3$)$_3$), 1.84 (s, 3H; C(5)CH$_3$), 2.12 (ddd, J=13.3, J=6.3, J=3.4 Hz, 1H; CH$_{2a}$(2′)), 2.17–2.22 (m, 1H; CH$_{2b}$(2′)), 3.57 (t, J=8.0 Hz, 2H; SiCH$_2$CH$_2$O), 3.72 (dd, J=11.4, J=3.8 Hz, 1H; CH$_{2a}$(5′)), 3.78 (dd, J=11.5, J=4.2 Hz, 1H; CH$_{2b}$(5′)), 3.81–3.85 (m, 1H; CH(4′)), 4.35–4.40 (m, 1H; CH(3′)), 5.22 (s, 2H; OCH$_2$N), 6.18 (t, J=6.5 Hz, 1H; CH(1′)), 7.51 ppm (s, 1H; CH(6)); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=−5.4 (2×SiCH$_3$), −4.8 (SiCH$_3$), −4.7 (SiCH$_3$), −1.3 (Si(CH$_3$)$_3$), 13.0 (C(5)CH$_3$), 17.6 (SiCH$_2$CH$_2$O), 17.8 (C(CH$_3$)$_3$), 18.1 (C(CH$_3$)$_3$), 25.8 (C(CH$_3$)$_3$), 25.9 (C(CH$_3$)$_3$), 39.6 (CH$_2$(2′)), 62.7 (CH$_2$(5′)), 66.5 (SiCH$_2$CH$_2$O), 69.8 (OCH$_2$N), 72.1 (CH(3′)), 85.1 (CH(1′)), 87.1 (CH(4′)), 108.9 (C(5)), 135.1 (CH(6)), 150.6 (CO), 162.8 ppm (CO); MS (FAB$^+$): m/z (%): 601 (17) [M+H$^+$], 543 (15), 229 (34), 213 (30), 199 (47), 145 (100), 115 (21).

N^3-Trimethylsilylethoxymethyl-3′,5′-*O*-di(*tert*-butydimethylsilyl)-5-bromomethyl-2′-deoxyuridine (7):[40] The protected thymidine **6** (1.00 g, 1.66 mmol), NBS (621 mg, 3.49 mmol), and benzoyl peroxide (12.0 mg, 0.05 mmol) were dissolved in carbon tetrachloride (20 mL). The reaction was heated at 70°C for 1 h. The reaction was allowed to cool and was filtered through a sintered funnel. The solvent was removed in vacuo to yield a crude yellow oil. Carrying out quick flash chromatography (silica gel, pentane/ethyl acetate 9:1) gave **7** as a colorless oil (683 mg, 60 %), which decomposed at room temperature but was stable at −20°C.

R_f=0.33 (pentane/ethyl acetate 9:1); ^{1}H NMR (300 MHz, CDCl$_3$): δ=0.00 (s, 9H; Si(CH$_3$)$_3$), 0.07 (s, 6H; Si(CH$_3$)$_2$), 0.13 (s, 6H; Si(CH$_3$)$_2$), 0.89 (s, 9H; C(CH$_3$)$_3$), 0.91 (s, 2H; SiCH$_2$CH$_2$O), 0.94 (s, 9H; C(CH$_3$)$_3$), 1.92–2.04 (m, 1H; CH$_{2a}$(2′)), 2.34 (ddd, J=8.6, J=5.6, J=2.7 Hz, 1H; CH$_{2b}$(2′)), 3.64–3.72 (m, 2H; SiCH$_2$CH$_2$O), 3.77 (dd, J=11.3, J=2.6 Hz, 1H; CH$_{2a}$(5′)), 3.89 (dd, J=11.6, J=2.6 Hz, 1H; CH$_{2b}$(5′)), 4.27 (d, J=10.3 Hz, 1H; CH$_{2a}$Br), 4.30 (d, J=10.3 Hz, 1H; CH$_{2b}$Br), 4.35–4.41 (m, 1H; CH(3′)), 5.41 (s, 2H; OCH$_2$N), 6.30 (dd, J=7.6, J=5.6 Hz, 1H; CH(1′)), 7.88 ppm (s, 1H; CH(6)); ^{13}C NMR (75 MHz, CDCl$_3$): δ=−5.4 (2×SiCH$_3$), −4.9 (SiCH$_3$), −4.7 (SiCH$_3$), −1.5 (Si(CH$_3$)$_3$), 18.0 (C(CH$_3$)$_3$), 18.1 (SiCH$_2$CH$_2$O), 18.4 (C(CH$_3$)$_3$), 25.7 (C(CH$_3$)$_3$), 26.0 (C(CH$_3$)$_3$), 26.1 (CH$_2$Br), 41.9 (CH$_2$(2′)), 63.0 (CH$_2$(5′)), 67.7 (SiCH$_2$CH$_2$O), 70.2 (OCH$_2$N), 72.2 (CH(3′)), 86.2 (CH(1′)), 88.2 (CH(4′)), 110.9 (C(5)), 137.8 (CH(6)), 150.4 (CO), 161.3 ppm (CO); MS (FAB$^+$): m/z (%): 751 (25), 679 (8) [M+H$^+$], 653 (13), 623 (18), 599 (13), 543 (6), 287 (10), 213 (23), 145 (100).

N^3(A/B)-Di(trimethylsilylethoxymethyl)-5-(α-thymidyl)-5,6-dihydrothymidine (8a/b): Dihydrothymidine **5** (446 mg, 0.74 mmol), azeotropically dried with anhydrous toluene, was dissolved in anhydrous THF (4 mL) and cooled to −78°C. A freshly prepared LDA solution (diisopropylamine (156 μL, 1.11 mmol), BuLi (0.70 mL, 1.60 M in hexane) in anhydrous THF (2 mL) left at 0°C for 1 h) was slowly added and the reaction mixture was stirred at −78°C for 2 h before the addition of **7** (500 mg, 0.74 mmol) dissolved in anhydrous THF (6 mL). The reaction mixture was stirred at −78°C for 1.5 h and at 0°C for 1.5 h. The reaction was quenched by adding aqueous sodium bicarbonate (12 mL) and the aqueous phase was extracted with CHCl$_3$ (3×20 mL). The collected extracts were dried (MgSO$_4$) and the solvent was removed in vacuo.

For deprotection of the OH groups, a TBAF solution was prepared by dissolving TBAF (2.33 g, 7.40 mmol) in anhydrous THF (10 mL) and adding molecular sieves (4 Å). The solution was stirred at RT for 1.5 h to remove traces of water. The crude mixture of the coupling was dissolved in anhydrous THF (20 mL) and added to the TBAF solution. The reaction mixture was stirred at RT for 1.5 h until all starting material had reacted. The mixture was filtered, diluted with CHCl$_3$ (100 mL), washed with aqueous sodium bicarbonate (150 mL), and the aqueous phase was extracted with CHCl$_3$ (3×100 mL). The collected organic phases were dried (MgSO$_4$) and the solvent was removed in vacuo. An initial purification was achieved by using flash chromatography (silica gel, CHCl$_3$/MeOH 10:1) to give **8a/b** as a mixture of diastereomers (328 mg, 60 %).

The diastereomers were separated by using rp-HPLC using a 250/10 Nucleosil 120-3 C8-column (Macherey–Nagel) with a water/acetonitrile gradient (0→6 % acetonitrile in 20 min, 6→9 % acetonitrile in 30 min, 9→100 % acetonitrile in 5 min; flow rate: 3 mL min^{-1}; detection wavelength: 250 nm). The mixture was dissolved in water/acetonitrile (30 mL, 1:1) and for each separation 1 mL of the solution was injected through a Rheodyne valve on the column. After HPLC purification, the *S* isomer **8a** (82.0 mg, 15 %) and the *R* isomer **8b** (58.0 mg, 11 %) were isolated as colorless oils.

*Isomer (S)-**8a***: R_f=0.30 (CHCl$_3$/MeOH 10:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=−0.06 (s, 9H; Si(CH$_3$)$_3$), −0.05 (s, 9H; Si(CH$_3$)$_3$), 0.73–0.84 (m, 4H; 2×SiCH$_2$CH$_2$O), 1.03 (s, 3H; C(5A)CH$_3$), 1.81 (ddd, J=13.1, J=6.0, J=2.5 Hz, 1H; CH$_{2a}$(2′A)), 1.96–2.08 (m, 2H; CH$_{2b}$(2′A), CH$_{2a}$(2′B)), 2.15 (ddd, J=13.1, J=6.0, J=3.3 Hz, 1H; CH$_{2b}$(2′B)), 2.32 (d, J=13.9 Hz, 1H; C(5B)CH$_{2a}$), 2.78 (d, J=13.7 Hz, 1H; C(5B)CH$_{2b}$), 3.14 (d, J=13.3 Hz, 1H; CH$_{2a}$(6A)), 3.19 (d, J=13.3 Hz, 1H; CH$_{2b}$(6A)), 3.38–3.41 (m, 3H; CH$_2$(5′A), H$_2$O), 3.43–3.48 (m, 2H; SiCH$_2$CH$_2$O(A)), 3.50–3.55 (m, 2H; SiCH$_2$CH$_2$O(B)), 3.56–3.59 (m, 2H; CH$_2$(5′B)), 3.61–3.64 (m, 1H; CH(4′A)), 3.79–3.83 (m, 1H; CH(4′B)), 4.11–4.15 (m, 1H; CH(3′A)), 4.23–4.27 (m, 1H; CH(3′B)), 4.96 (d, J=9.6 Hz, 1H; OCH$_{2a}$N(A)), 5.04 (d, J=9.9 Hz, 1H; OCH$_{2b}$N(A)), 5.15 (d, J=9.9 Hz, 1H; OCH$_{2a}$N(B)), 5.17 (d, J=9.9 Hz, 1H; OCH$_{2b}$N(B)), 6.15–6.22 (m, 2H; 2×CH(1′)), 7.84 ppm (s, 1H; CH(6B)); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=−1.2 (2×Si(CH$_3$)$_3$), 17.6 (SiCH$_2$CH$_2$O), 17.8 (SiCH$_2$CH$_2$O), 20.0 (C(5A)CH$_3$), 31.7 (C(5B)CH$_2$), 36.2 (CH$_2$(2′A)), 40.1 (CH$_2$(2′B)), 41.4 (C(5A)), 44.4 (CH$_2$(6A)), 61.4 (CH$_2$(5′B)), 62.0 (CH$_2$(5′A)), 65.7 (SiCH$_2$CH$_2$O(A)), 66.5 (SiCH$_2$CH$_2$O(B)), 69.7 (OCH$_2$N(A)), 70.0 (OCH$_2$N(B)), 70.6 (CH(3′B)), 70.9 (CH(3′A)), 84.0 (CH(1′A)), 85.3 (CH(1′B)), 86.3 (CH(4′A)), 87.8 (CH(4′B)), 107.6 (C(5B)), 138.6 (CH(6B)), 150.4 (CO(2B)), 152.4 (CO(2A)), 163.0 (CO(4B)), 173.5 ppm (CO(4A)); IR (film): $\tilde{\nu}_{max}$=3400 s, 2952 s, 1710 s, 1666 s, 1465 s, 1394 w, 1365 w, 1338 w, 1279 m, 1247 m, 1199 m, 1091 s, 998 s, 917 w, 860 s, 837 s, 766 m, 694 w, 619 w cm^{-1}; MS (MALDI$^+$): m/z (%): 783 (17) [M+K$^+$], 767 (86) [M+Na$^+$], 306 (27), 284 (100), 215 (17), 175 (12); HRMS (ESI$^+$): m/z calcd for $C_{32}H_{56}N_4NaO_{12}Si_2$: 767.3331; found: 767.3296 [M+Na$^+$].

*Isomer (R)-**8b***: R_f=0.30 (CHCl$_3$/MeOH 10:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=−0.06 (s, 9H; Si(CH$_3$)$_3$), −0.04 (s, 9H; Si(CH$_3$)$_3$), 0.76–0.85 (m, 4H; 2×SiCH$_2$CH$_2$O), 1.02 (s, 3H; C(5A)CH$_3$), 1.77–1.84 (m, 1H; CH$_{2a}$(2′A)), 1.96–2.04 (m, 1H; CH$_{2b}$(2′A)), 2.07–2.14 (m, 2H; CH$_2$(2′B)), 2.19 (d, J=14.2 Hz, 1H; C(5B)CH$_{2a}$), 2.83 (d, J=13.9 Hz, 1H; C(5B)CH$_{2b}$), 3.11 (d, J=13.2 Hz, 1H; CH$_{2a}$(6A)), 3.31 (d, J=13.1 Hz, 1H; CH$_{2b}$(6A)), 3.41–3.48 (m, 4H; CH$_2$(5′A), SiCH$_2$CH$_2$O(A)), 3.49–3.61 (m, 4H; CH$_2$(5′B), SiCH$_2$CH$_2$O(B)), 3.63–3.68 (m, 1H; CH(4′A)), 3.77–3.82 (m, 1H; CH(4′B)), 4.11–4.16 (m, 1H; CH(3′A)), 4.22–4.27 (m, 1H; CH(3′B)), 4.75 (t, J=5.4 Hz, 1H; OH(5′A)), 4.92 (d, J=9.8 Hz, 1H; OCH$_{2a}$N(A)), 4.98 (t, J=5.0 Hz, 1H; OH(5′B)), 5.07 (d, J=9.9 Hz, 1H; OCH$_{2b}$N(A)), 5.10–5.19 (m, 3H; OH(3′A), OCH$_2$N(B)), 5.26 (d, J=4.1 Hz, 1H; OH(3′B)), 6.17–6.20 (m, 2H; 2×CH(1′)), 7.73 ppm (s, 1H; CH(6B)); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=−1.2 (2×Si(CH$_3$)$_3$), 17.5 (SiCH$_2$CH$_2$O), 17.8 (SiCH$_2$CH$_2$O), 19.6 (C(5A)CH$_3$), 32.2 (C(5B)CH$_2$), 36.1 (CH$_2$(2′A)), 41.3 (C(5A)), 44.7 (CH$_2$(6A)), 61.5 (CH$_2$(5′B)), 62.2 (CH$_2$(5′A)), 65.6 (SiCH$_2$CH$_2$O(A)), 66.5 (SiCH$_2$CH$_2$O(B)), 69.6 (OCH$_2$N(A)), 70.0 (OCH$_2$N(B)), 70.6 (CH(3′B)), 71.0 (CH(3′A)), 83.9

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(CH(1′A)), 85.1 (CH(1′B)), 86.4 (CH(4′A)), 87.7 (CH(4′B)), 107.9 (C(5B)), 138.5 (CH(6B)), 150.5 (CO(2B)), 152.1 (CO(2A)), 162.9 (CO(4B)), 173.3 ppm (CO(4A)); IR (film): $\tilde{\nu}_{max}$ = 3442 s, 2952 s, 1653 s, 1465 m, 1364 w, 1278 w, 1247 w, 1200 w, 1091 m, 916 w, 860 m, 836 m, 760 m, 694 w, 667 w cm^{-1}; MS (MALDI$^+$): m/z (%): 783 (3) [M+K$^+$], 767 (29) [M+Na$^+$], 306 (30), 253 (6), 242 (100), 197 (5); HRMS (ESI$^+$): m/z calcd for $C_{32}H_{56}N_4NaO_{12}Si_2$: 767.3331; found: 767.3343 [M+Na]$^+$.

(5*R*)-(α-Thymidyl)-5,6-dihydrothymidine (1b): Isomer (*R*)-**8b** (59.0 mg, 0.08 mmol) was dissolved in anhydrous CH_2Cl_2 (7 mL) and the solution was cooled to 0°C. A solution of $SnCl_4$ (0.80 mL, 1 M in CH_2Cl_2) was added dropwise. The reaction mixture was stirred at 0°C for 1 h. The reaction was quenched by adding methanolic sodium hydroxide (3 mL, 4% NaOH). The solvent was removed under reduced pressure. The crude product was suspended in methanol (4 mL), and centrifuged and decanted to remove the tin salts. This procedure was repeated twice. The solvent was removed from the collected solutions in vacuo. Purification by using flash chromatography (silica gel, $CHCl_3$/MeOH 4:1) provided **1b** as a white solid (27.0 mg, 75%).

R_f = 0.10 ($CHCl_3$/MeOH 4:1); m.p. 155–158°C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 0.98 (s, 3H; C(5A)CH_3), 1.77 (ddd, J = 12.8, J = 6.2, J = 2.5 Hz, 1H; CH_{2a}(2′A)), 1.94–2.01 (m, 1H; CH_{2b}(2′A)), 2.05–2.11 (m, 2H; CH_2(2′B)), 2.25 (d, J = 14.2 Hz, 1H; C(5B)CH_{2a}), 2.73 (d, J = 13.3 Hz, 1H; C(5B)CH_{2b}), 3.05 (d, J = 13.1 Hz, 1H; CH_{2a}(6A)), 3.23 (d, J = 13.1 Hz, 1H; CH_{2b}(6A)), 3.41 (t, J = 5.3 Hz, 2H; CH_2(5′A)), 3.51–3.57 (m, 2H; CH_2(5′B)), 3.59–3.63 (m, 1H; CH(4′A)), 3.74–3.79 (m, 1H; CH(4′B)), 4.07–4.12 (m, 1H; CH(3′A)), 4.21–4.27 (m, 1H; CH(3′B)), 4.81 (t, J = 5.5 Hz, 1H; OH(5′A)), 5.02 (t, J = 5.3 Hz, 1H; OH(5′B)), 5.30 (d, J = 4.1 Hz, 1H; OH(3′A)), 5.30 (d, J = 4.1 Hz, 1H; OH(3′B)), 6.08–6.17 (m, 2H; 2×CH(1′)), 7.65 (s, 1H; CH(6B)), 10.05 (s, 1H; NH(A)), 11.22 ppm (s, 1H; NH(B)); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 19.5 (C(5A)CH$_3$), 31.1 (C(5B)CH$_2$), 36.0 (CH$_2$(2′A)), 41.2 (C(5A)), 46.0 (CH$_2$(6A)), 61.7 (CH$_2$(5′B)), 62.2 (CH$_2$(5′A)), 70.8 (CH(3′B)), 71.0 (CH(3′A)), 83.1 (CH(1′A)), 84.3 (CH(1′B)), 86.2 (CH(4′A)), 87.6 (CH(4′B)), 108.8 (C(5B)), 139.1 (CH(6B)), 150.4 (CO(2B)), 152.4 (CO(2A)), 163.7 (CO(4B)), 174.2 ppm (CO(4A)); IR (KBr): $\tilde{\nu}_{max}$ = 3419 s, 2924 m, 2854 w, 1697 s, 1476 m, 1389 w, 1278 w, 1205 m, 1091 m, 1050 m, 766 w cm^{-1}; MS (MALDI$^+$): m/z (%): 507 (100) [M+Na$^+$], 444 (5), 412 (5), 390 (6), 314 (10), 306 (46), 288 (29), 284 (35), 268 (15), 254 (12), 165 (13); HRMS (ESI$^+$): m/z calcd for $C_{20}H_{28}N_4NaO_{10}$: 507.1703; found: 507.1694 [M+Na]$^+$; m/z calcd for $C_{20}H_{28}KN_4O_{10}$: 523.1443; found: 523.1434 [M+K]$^+$.

(5*S*)-(α-Thymidyl)-5,6-dihydrothymidine (1a): SEM deprotection was carried out as described above for **1b**. Isomer (*S*)-**1a** was isolated as a white solid (27.0 mg, 55%).

R_f = 0.10 ($CHCl_3$/MeOH 4:1); m.p. 155–158°C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 0.99 (s, 3H; C(5A)CH_3), 1.77 (ddd, J = 13.2, J = 6.2, J = 2.8 Hz, 1H; CH_{2a}(2′A)), 1.94–2.06 (m, 2H; CH_{2b}(2′A), CH_{2a}(2′B)), 2.10 (ddd, J = 13.2, J = 5.9, J = 3.1 Hz, 1H; CH_{2b}(2′B)), 2.30 (d, J = 13.8 Hz, 1H; C(5B)CH_{2a}), 2.72 (d, J = 14.0 Hz, 1H; C(5B)CH_{2b}), 3.05 (d, J = 12.9 Hz, 1H; CH_{2a}(6A)), 3.17 (d, J = 13.0 Hz, 1H; CH_{2b}(6A)), 3.36 (s, 13H; CH_2(5′A), H_2O), 3.51–3.59 (m, 3H; CH_2(5′B), CH(4′A)), 3.75–3.79 (m, 1H; CH(4′B)), 4.08–4.13 (m, 1H; CH(3′A)), 4.23–4.28 (m, 1H; CH(3′B)), 4.77 (t, J = 5.5 Hz, 1H; OH(5′A)), 5.14 (t, J = 5.1 Hz, 1H; OH(5′B)), 5.19 (d, J = 4.2 Hz, 1H; OH(3′A)), 5.34 (d, J = 4.2 Hz, 1H; OH(3′B)), 6.09 (dd, J = 7.9, J = 6.2 Hz, 1H; CH(1′A)), 6.13 (t, J = 7.2 Hz, 1H; CH(1′B)), 7.72 (s, 1H; CH(6B)), 10.11 (s, 1H; NH(A)), 11.23 ppm (s, 1H; NH(B)); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 20.2 (C(5A)CH$_3$), 30.8 (C(5B)CH$_2$), 35.9 (CH$_2$(2′A)), 41.1 (C(5A)), 45.5 (CH$_2$(6A)), 61.50 (CH$_2$(5′B)), 62.0 (CH$_2$(5′A)), 70.7 (CH(3′B)), 70.8 (CH(3′A)), 83.0 (CH(1′A)), 84.2 (CH(1′B)), 86.0 (CH(4′A)), 87.6 (CH(4′B)), 108.7 (C(5B)), 139.1 (CH(6B)), 150.4 (CO(2B)), 152.5 (CO(2A)), 163.8 (CO(4B)), 174.4 ppm (CO(4A)); IR (KBr): $\tilde{\nu}_{max}$ = 3393 s, 2918 m, 2253 w, 1693 s, 1474 w, 1389 w, 1278 w, 1232 w, 1093 m, 1051 m, 1026 m, 1002 m, 827 w, 766 w, 574 w cm^{-1}; MS (ESI$^+$): m/z (%): 523 (57) [M+K$^+$], 407 (16), 336 (12), 303 (100), 257 (17), 122 (45); HRMS (ESI$^+$): m/z calcd for $C_{20}H_{28}KN_4O_{10}$: 523.1443; found: 523.1450 [M+K]$^+$.

5′-*O*-*tert*-Butyldimethylsilyl-5,6-dihydrothymidine (11):[37–39] Thymidine **3** (2.50 g, 10.3 mmol) was dissolved in MeOH/water (50 mL, 1:1) and Rh/Al$_2$O$_3$ (100 mg, 5% Rh) was added. The suspension was stirred under an H$_2$ atmosphere at RT for 2 d. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure. The crude product was azeotropically dried three times with pyridine and dissolved in anhydrous DMF (11 mL). Imidazole (1.54 g, 22.7 mmol) and TBDMSCl (1.71 g, 11.3 mmol) were added and the reaction mixture was stirred for 1 h at RT. The reaction mixture was diluted with $CHCl_3$ (50 mL), washed with saturated aqueous sodium bicarbonate (7×100 mL), dried (MgSO$_4$), and the solvent was removed in vacuo. Purification by using flash chromatography (silica gel, $CHCl_3$/MeOH 20:1) provided **11** as a white foam (3.01 g, 82%).

R_f = 0.20 ($CHCl_3$/MeOH 20:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.04 (s, 3H; Si(CH_3)$_{2a}$), 0.05 (s, 3H; Si(CH_3)$_{2b}$), 0.87 (s, 9H; C(CH_3)$_3$), 1.06 (d, J = 7.2 Hz; 3H, CH_3), 1.79–1.85 (m, 1H; CH_{2a}(2′)), 2.03–2.10 (m, 1H; CH_{2b}(2′)), 2.53–2.64 (m, 1H; CH(5)), 2.99 (dd, J = 12.4, J = 10.0 Hz, 1H; CH_{2a}(6)), 3.38 (dd, J = 12.8, J = 5.6 Hz, 1H; CH_{2b}(6)), 3.60–3.67 (m, 3H; CH(4′), CH_2(5′)), 4.10–4.11 (m, 1H; CH(3′)), 5.13–5.14 (m, 1H; OH), 6.11–6.14 (m, 1H; CH(1′)), 10.19 ppm (s, 1H; NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −5.5 (SiCH$_3$), −5.5 (SiCH$_3$), 12.6 (CH$_3$), 17.9 (C(CH$_3$)$_3$), 25.8 (C(CH$_3$)$_3$), 34.6 (CH(5)), 36.4 (CH$_2$(2′)), 41.3 (CH$_2$(6)), 63.3 (CH$_2$(5′)), 70.3 (CH(3′)), 82.7 (CH(1′)), 85.3 (CH(4′)), 152.8 (CO), 173.0 ppm (CO); MS (FAB$^+$): m/z: 740 [2M+H$^+$], 381 [M+Na$^+$], 359 [M+H$^+$]; HRMS (FAB$^+$): m/z calcd for $C_{16}H_{31}N_2O_5Si$: 359.2002; found: 359.2002 [M+H]$^+$.

3′-*O*-Triethylsilyl-5′-*O*-*tert*-butyldimethylsilyl-5,6-dihydrothymidine (18): The TBDMS-protected dihydrothymidine **11** (2.97 g, 8.29 mmol), imidazole (1.69 g, 24.9 mmol), and TESCl (2.09 mL, 12.4 mmol) were dissolved in anhydrous DMF (13 mL). The reaction mixture was stirred overnight at RT. The solution was diluted with $CHCl_3$ (60 mL), washed with saturated aqueous sodium bicarbonate (7×120 mL), dried (MgSO$_4$), and the solvent was removed in vacuo. Carrying out flash chromatography (silica gel, *i*-hexane/ethyl acetate 7:3) gave **18** as a white foam (3.50 g, 89%).

R_f = 0.31 (*i*Hex/ethyl acetate 7:3); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.05 (s, 3H; Si(CH_3)$_{2a}$), 0.06 (s, 3H; Si(CH_3)$_{2b}$), 0.59 (q, J = 8.0 Hz, 6H; Si(CH_2CH$_3$)$_3$), 0.87 (s, 9H; C(CH_3)$_3$), 0.93 (t, J = 8.0 Hz, 9H; Si(CH$_2$CH_3)$_3$)), 1.06 (d, J = 7.2 Hz, 3H; CH_3), 1.78–1.83 (m, 1H; CH_{2a}(2′)), 2.14–2.21 (m, 1H; CH_{2b}(2′)), 2.56–2.66 (m, 1H; CH(5)), 2.97 (dd, J = 12.4, J = 10.4 Hz, 1H; CH_{2a}(6)), 3.37 (dd, J = 12.8, J = 5.6 Hz, 1H; CH_{2b}(6)), 3.60–3.66 (m, 3H; CH(4′), CH_2(5′)), 4.27–4.29 (m, 1H; CH(3′)), 6.10–6.14 (dd, J = 6.4, J = 6.4 Hz, 1H; CH(1′)), 10.22 ppm (s, 1H; NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −5.6 (SiCH$_3$), −5.6 (SiCH$_3$), 4.2 (Si(CH$_2$CH$_3$)$_3$), 6.5 (Si(CH$_2$CH$_3$)$_3$), 12.5 (CH$_3$), 17.9 (C(CH$_3$)$_3$), 25.7 (C(CH$_3$)$_3$), 34.5 (CH(5)), 36.3 (CH$_2$(2′)), 41.4 (CH$_2$(6)), 62.7 (CH$_2$(5′)), 71.7 (CH(3′)), 82.8 (CH(1′)), 85.3 (CH(4′)), 152.9 (CO), 173.0 ppm (CO); MS (FAB$^+$): m/z: 969 [2M+Na$^+$], 496 [M+Na$^+$], 474 [M+H$^+$]; HRMS (MALDI$^+$): m/z calcd for $C_{22}H_{44}N_2NaO_5Si_2$: 495.2686; found: 495.2637 [M+Na]$^+$.

***N*^{3}-Trimethylsilylethoxymethyl-3′-*O*-triethylsilyl-5′-*O*-*tert*-butyldimethylsilyl-5,6-dihydrothymidine (10)**:[16] SEM protection was carried out as described above for **5**. Product **10** (3.80 g, 86%) was isolated as a colorless oil.

R_f = 0.19 (*i*Hex/ethyl acetate 9:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = −0.04 (s, 9H; Si(CH_3)$_3$), 0.05 (s, 3H; Si(CH_3)$_{2a}$), 0.06 (s, 3H; Si(CH_3)$_{2b}$), 0.59 (q, J = 8.0 Hz, 6H; Si(CH_2CH$_3$)$_3$), 0.79–0.84 (m, 2H; SiCH_2CH$_2$O), 0.87 (s, 9H; C(CH_3)$_3$), 0.93 (t, J = 8.0 Hz, 9H; Si(CH$_2$CH_3)$_3$), 1.10 (d, J = 7.2 Hz, 3H; CH_3), 1.82–1.88 (m, 1H; CH_{2a}(2′)), 2.15–2.22 (m, 1H; CH_{2b}(2′)), 2.73–2.78 (m, 1H; CH(5)), 3.00 (dd, J = 12.8, J = 10.0 Hz, 1H; CH_{2a}(6)), 3.38 (dd, J = 12.8, J = 5.6 Hz, 1H; CH_{2b}(6)), 3.50 (t, J = 8.0 Hz, 2H; SiCH$_2$CH_2O), 3.62–3.68 (m, 3H; CH(4′), CH_2(5′)), 4.28–4.31 (m, 1H; CH(3′)), 5.05 (s, 2H; OCH_2N), 6.15–6.18 ppm (dd, J = 6.4, J = 6.4 Hz, 1H; CH(1′)); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −5.7 (SiCH$_3$), −5.6 (SiCH$_3$), −1.4 (Si(CH$_3$)$_3$), 4.1 (Si(CH$_2$CH$_3$)$_3$), 6.5 (Si(CH$_2$CH$_3$)$_3$), 12.9 (CH$_3$), 17.4 (OCH$_2$CH$_2$Si), 17.9 (C(CH$_3$)$_3$), 25.6 (C(CH$_3$)$_3$), 34.9 (CH(5)), 36.5 (CH$_2$(2′)), 40.2 (CH$_2$(6)), 62.6 (CH$_2$(5′)), 65.5 (OCH$_2$CH$_2$Si), 69.0 (NCH$_2$O), 71.5 (CH(3′)), 83.7(CH(1′)), 85.4 (CH(4′)), 152.6 (CO), 172.3 ppm (CO); MS (FAB$^+$): m/z: 604 [M+H$^+$]; HRMS (MALDI$^+$): m/z calcd for $C_{28}H_{58}N_2NaO_6Si_3$: 625.3500; found: 625.3496 [M+Na]$^+$.

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3′-*O*-*tert*-Butyldimethylsilylthymidine (13):[41] The bis-TBDMS-protected thymidine **17** (3.53 g, 7.50 mmol) was dissolved in CH_2Cl_2 (41 mL) and cooled to 0 °C. A TFA/water mixture (4.1 mL, 10:1, cooled to 0 °C) was added dropwise to the solution. The reaction mixture was stirred for 4 h at 0 °C. The solution was diluted with cooled CH_2Cl_2 (9 mL), washed with ice-cold water (50 mL) and with saturated aqueous sodium chloride (25 mL), dried ($MgSO_4$), and the solvent was removed in vacuo. Carrying out flash chromatography (silica gel, $CHCl_3$/MeOH 20:1) gave **13** as a white foam (2.17 g, 81 %).

R_f = 0.18 ($CHCl_3$/MeOH 20:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.08 (s, 6 H; Si(CH_3)$_2$), 0.87 (s, 9 H; C(CH_3)$_3$), 1.77 (s, 3 H; CH_3), 2.00–2.06 (m, 1 H; CH_{2a}(2′)), 2.15–2.21 (m, 1 H; CH_{2b}(2′)), 3.51–3.61 (m, 2 H; CH_2(5′)), 3.74–3.77 (m, 1 H; CH(4′)), 4.39–4.42 (m, 1 H; CH(3′)), 5.06–5.08 (m, 1 H; OH), 6.13–6.16 (m, 1 H; CH(1′)), 7.66 (s, 1 H; CH(6)), 11.28 ppm (s, 1 H; NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −4.9 (SiCH$_3$), −4.8 (SiCH$_3$), 12.2 (CH$_3$), 17.7 (C(CH$_3$)$_3$), 25.6 (C(CH$_3$)$_3$), 39.5 (CH$_2$(2′)), 60.9 (CH$_2$(5′)), 72.1 (CH(3′)), 83.7 (CH(1′)), 87.3 (CH(4′)), 109.4 (C(5)), 136.0 (CH(6)), 150.4 (CO), 163.6 ppm (CO); MS (FAB$^+$): m/z: 714 [2M+H$^+$], 379 [M+Na$^+$], 357 [M+H$^+$], 117 [TBS+H$^+$].

3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-triethylsilylthymidine (19): TES protection was carried out as described above for **18**. Product **19** was isolated as a colorless oil (2.53 g, 91 %).

R_f = 0.36 (*i*Hex/ethyl acetate 7:3); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.08 (s, 6 H; Si(CH_3)$_2$), 0.60 (q, J = 8.0 Hz, 6 H; Si(CH_2CH$_3$)$_3$), 0.87 (s, 9 H; C(CH_3)$_3$), 0.93 (t, J = 8.0 Hz, 9 H; Si(CH$_2$CH_3)$_3$)), 1.78 (s, 3 H; CH_3), 2.03–2.09 (m, 1 H; CH_{2a}(2′)), 2.17–2.24 (m, 1 H; CH_{2b}(2′)), 3.67–3.75 (m, 2 H; CH_2(5′)), 3.76–3.80 (m, 1 H; CH(4′)), 4.36–4.39 (m, 1 H; CH(3′)), 6.14–6.17 (dd, J = 6.4, J = 6.4 Hz, 1 H; CH(1′)), 7.47 (s, 1 H; CH(6)), 11.32 ppm (s, 1 H; NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −5.0 (SiCH$_3$), −4.9 (SiCH$_3$), 3.8 (Si(CH$_2$CH$_3$)$_3$), 6.5 (Si(CH$_2$$CH_3$)$_3$), 12.1 ($CH_3$), 17.6 ($C$(CH$_3$)$_3$), 25.6 (C($CH_3$)$_3$), 62.2 ($CH_2$(5′)), 72.0 ($C$H(3′)), 83.7 ($C$H(1′)), 86.7 ($C$H(4′)), 109.4 ($C$(5)), 135.6 ($C$H(6)), 150.3 ($C$O), 163.6 ppm ($C$O); MS (FAB$^+$): m/z: 494 [M+Na$^+$], 472 [M+H$^+$], 117 [TES+H$^+$] and [TBS+H$^+$]; HRMS (MALDI$^+$): m/z calcd for $C_{22}H_{42}N_2NaO_5Si_2$: 493.2530; found: 493.2541 [M+Na]$^+$.

***N*^{3}-Trimethylsilylethoxymethyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilylthymidine (14)**: SEM protection was carried out as described above for **5**. Product **14** was isolated as a colorless oil (2.04 g, 64 %).

R_f = 0.24 (*i*Hex/ethyl acetate 9:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = −0.05 (s, 9 H; Si(CH$_3$)$_3$), 0.08 (s, 6 H; Si(CH_3)$_2$), 0.60 (q, J = 7.9 Hz, 6 H; Si(CH_2CH$_3$)$_3$), 0.83 (t, J = 8.0 Hz, 2 H; SiCH_2CH$_2$O), 0.87 (s, 9 H; C-(CH_3)$_3$), 0.92 (t, ^3J = 8.0 Hz, 9 H; Si(CH$_2$CH_3)$_3$), 1.83 (s, 3 H; CH_3), 2.08–2.14 (m, 1 H; CH_{2a}(2′)), 2.18–2.25 (m, 1 H; CH_{2b}(2′)), 3.56 (t, J = 8.0 Hz, 2 H; SiCH$_2$CH_2O), 3.71 (dd, J = 11.2, J = 3.6 Hz, 1 H; CH_{2a}(5′)), 3.77 (dd, J = 11.2, J = 4.0 Hz, 1 H; CH_{2b}(5′)), 3.80–3.83 (m, 1 H; CH(4′)), 4.36–4.39 (m, 1 H; CH(3′)), 5.21 (s, 2 H; OCH_2N), 6.15–6.19 (m, 1 H; CH(1′)), 7.56 ppm (s, 1 H; CH(6)); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −5.0 (SiCH$_3$), −4.9 (SiCH$_3$), −1.5 (Si(CH$_3$)$_3$), 3.8 (Si(CH$_2$CH$_3$)$_3$), 6.5 (Si-(CH$_2$$CH_3$)$_3$), 12.7 ($CH_3$), 17.4 (Si$CH_2CH_2$O), 17.6 ($C$(CH$_3$)$_3$), 25.6 (C-($CH_3$)$_3$), 39.4 ($CH_2$(2′)), 62.1 ($CH_2$(5′)), 66.3 (SiCH$_2$$CH_2$O), 69.6 (O$CH_2$N), 71.8 ($C$H(3′)), 84.9 ($C$H(1′)), 86.9 ($C$H(4′)), 108.6 ($C$(5)), 134.9 ($C$H(6)), 150.4 ($C$O), 162.6 ppm ($C$O); MS (FAB$^+$): m/z: 1225 [2M+H$^+$], 624 [M+Na$^+$], 602 [M+H$^+$], 117 [TES+H$^+$] and [TBS+H$^+$]; HRMS (MALDI$^+$): m/z calcd for $C_{28}H_{56}N_2NaO_6Si_3$: 623.3344; found: 623.3319 [M+Na]$^+$.

***N*^{3}-Trimethylsilylethoxymethyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-triethylsilyl-5-bromomethyl-2′-deoxyuridine (12)**:[40] The bromination was carried out as described above for **7**. Product **12** was isolated as a colorless oil (112 mg, 20 %), which decomposed at room temperature but was stable at −20 °C.

R_f = 0.30 (*i*Hex/ethyl acetate 9:1); ^{1}H NMR (200 MHz, CDCl$_3$): δ = 0.00 (s, 9 H; Si(CH_3)$_3$), 0.07 (s, 6 H; Si(CH_3)$_2$), 0.64 (q, J = 8.0 Hz, 6 H; Si-(CH_2CH$_3$)$_3$), 0.89–0.93 (m, 11 H; SiCH_2CH$_2$O, C(CH_3)$_3$), 0.97–1.04 (m, 9 H; Si(CH$_2$CH_3)$_3$), 1.94–2.09 (m, 1 H; CH_{2a}(2′)), 2.29–2.40 (m, 1 H; CH_{2b}(2′)), 3.64–3.72 (m, 2 H; SiCH$_2$CH_2O), 3.79–3.98 (m, 3 H; CH(4′), CH_2(5′)), 4.23 (d, J = 10.6 Hz, 1 H; CH_{2a}Br), 4.32 (d, J = 10.6 Hz, 1 H; CH_{2b}Br), 4.39–4.42 (m, 1 H; CH(3′)), 5.41 (s, 2 H; OCH_2N), 6.28–6.34 (m, 1 H; CH(1′)), 7.97 ppm (s, 1 H; CH(6)).

^{13}C NMR and mass spectral data were unobtainable due to the rapid decomposition of **12**.

***N*^{3}(A/B)-Di(trimethylsilylethoxymethyl)-5′(A),3′(B)-*O*-di(*tert*-butyldimethylsilyl)-5-(α-thymidyl)-5,6-dihydrothymidine (15 a/b)**: Dihydrothymidine **10** (87 mg, 0.14 mmol), azeotropically dried with anhydrous toluene, was dissolved in anhydrous THF (0.8 mL) and cooled to −78 °C. A freshly prepared LDA solution (diisopropylamine (31 μL, 0.22 mmol), BuLi (0.14 mL, 1.60 M in hexane) in anhydrous THF (0.4 mL) left at 0 °C for 1 h) was slowly added and the reaction mixture was stirred at −78 °C for 2 h before the addition of **12** (100 mg, 0.15 mmol), dissolved in anhydrous THF (1.2 mL). The reaction mixture was stirred at −78 °C for 1.5 h and at 0 °C for 1.5 h. The reaction was quenched by adding aqueous sodium bicarbonate (5 mL) and the aqueous phase was extracted with CHCl$_3$ (3 × 5 mL). The collected extracts were dried ($MgSO_4$) and the solvent was removed in vacuo.

The crude mixture of the coupling reaction was dissolved in anhydrous acetonitrile (1.29 mL) and cooled to −30 °C. A HF•pyridine solution (4 % in acetonitrile, 2.92 mL, 937 μmol) was added and the reaction mixture was stirred at −30 °C for 40 min. The reaction was quenched by adding methoxytrimethylsilane, stirred at −30 °C for 1 h, and the solvent was removed in vacuo. An initial purification was achieved by using flash chromatography (silica gel, *i*-hexane/ethyl acetate 4:1→3:2) to give **9 a/b** as colorless oil (48.0 mg, 34 %).

The diastereomers were separated by using np-HPLC with a 250/10 Nucleodur 100-5 column (Macherey–Nagel) and an *n*-heptane/ethyl acetate gradient (0→70 % ethyl acetate in 70 min, 70→100 % ethyl acetate in 5 min; flow rate: 3 mL min^{-1}; detection wavelength: 260 nm). The mixture was dissolved in *n*-heptane/ethyl acetate (9 mL, 1:1) and for each separation 1 mL of the solution was injected through a Rheodyne valve on the column. After HPLC purification, the isomers (*S*)-**9 a** (13.0 mg, 9 %) and (*R*)-**9 b** (18.0 mg, 13 %) were isolated as colorless oils.

*Isomer (S)-**9 a***: R_f = 0.45 (*i*Hex/ethyl acetate 3:2); ^{1}H NMR (400 MHz, CDCl$_3$): δ = −0.02 (s, 9 H; Si(CH_3)$_3$), −0.01 (s, 9 H; Si(CH_3)$_3$), 0.06 (s, 3 H; Si(CH_3)), 0.07 (s, 3 H; Si(CH_3)), 0.11 (s, 6 H; 2 × Si(CH_3)), 0.88–0.91 (m, 22 H; 2 × SiCH_2CH$_2$O, 2 × C(CH_3)$_3$), 1.22 (s, 3 H; CH_3), 2.00–2.18 (m, 3 H; CH_2(2′A), CH_{2a}(2′B)), 2.36 (ddd, J = 13.2, J = 6.0, J = 2.8 Hz, 1 H; CH_{2b}(2′B)), 2.57 (d, J = 14.0 Hz, 1 H; C(5B)CH_{2a}), 2.82 (d, J = 14.0 Hz, 1 H; C(5B)CH_{2b}), 3.13 (d, J = 12.8 Hz, 1 H; CH_{2a}(6A)), 3.23 (d, J = 12.8 Hz, 1 H; CH_{2b}(6A)), 3.57–3.69 (m, 5 H; 2 × SiCH$_2$CH_2O, CH_{2a}(5′A)), 3.71 (dd, J = 12.0, J = 2.4 Hz, 1 H; CH_{2a}(5′B)), 3.76–3.79 (m, 1 H; CH-(4′A)), 3.83 (dd, J = 10.0, J = 4.0 Hz, 1 H; CH_{2b}(5′A)), 3.97 (dd, J = 12.0, J = 2.4 Hz, 1 H; CH_{2b}(5′B)), 4.01–4.03 (m, 1 H; CH(4′B)), 4.34–4.38 (m, 1 H; CH(3′A)), 4.44–4.46 (m, 1 H; CH(3′B)), 5.13 (d, J = 9.6 Hz, 1 H; OCH_{2a}N(A)), 5.17 (d, J = 9.6 Hz, 1 H; OCH_{2b}N(A)), 5.34 (d, J = 9.6 Hz, 1 H; OCH_{2a}N(B)), 5.37 (d, J = 9.6 Hz, 1 H; OCH_{2b}N(B)), 6.14–6.18 (m, 1 H; CH(1′B)), 6.31–6.34 (m, 1 H; CH(1′A)), 7.88 ppm (s, 1 H; CH(6B)); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = −5.2 (SiCH$_3$), −5.2 (SiCH$_3$), −4.7 (SiCH$_3$), −4.5 (SiCH$_3$), −1.29 (Si(CH$_3$)$_3$), −1.25 (Si(CH$_3$)$_3$), 18.1 (C-(CH$_3$)$_3$), 18.2 (SiCH$_2$CH$_2$O), 18.3 (SiCH$_2$CH$_2$O), 18.5 (C(CH$_3$)$_3$), 22.0 (CH$_3$), 25.9 (C(CH$_3$)$_3$), 26.2 (C(CH$_3$)$_3$), 32.2 (C(5B)CH$_2$), 36.7 (CH$_2$-(2′A)), 42.2 (CH$_2$(2′B)), 43.1 (CH(5A)), 45.1 (CH$_2$(6A)), 62.3 (CH$_2$(5′B)), 64.1 (CH$_2$(5′A)), 67.3 (SiCH$_2$$CH_2$O(A)), 67.7 (SiCH$_2$$CH_2$O(B)), 70.2 (O$CH_2$N(A)), 70.4 (O$CH_2$N(B)), 72.7 ($C$H(3′B)), 73.0 ($C$H(3′A)), 84.3 ($C$H(1′A)), 84.8 ($C$H(4′A)), 87.9 ($C$H(1′B)), 88.9 ($C$H(4′B)), 107.7 ($C$(5B)), 139.9 ($C$H(6B)), 150.7 ($C$O(2B)), 152.4 ($C$O(2A)), 164.0 ($C$O(4B)), 174.5 ppm ($C$O(4A)); MS (MALDI$^+$): m/z: 1012 [M+K$^+$], 996 [M+Na$^+$]; HRMS (MALDI$^+$): m/z calcd for $C_{44}H_{83}N_4NaO_{12}Si_4$: 994.4982; found: 994.4942 [M+Na]$^+$.

*Isomer (R)-**9 b***: R_f = 0.45 (*i*Hex/ethyl acetate 3:2); ^{1}H NMR (400 MHz, CDCl$_3$): δ = −0.03 (s, 9 H; Si(CH_3)$_3$), −0.02 (s, 9 H; Si(CH_3)$_3$), 0.06 (s, 3 H; SiCH_3), 0.08 (s, 3 H; SiCH_3), 0.10 (s, 3 H; SiCH_3), 0.11 (s, 3 H; SiCH_3), 0.88–0.91 (m, 22 H; 2 × SiCH_2CH$_2$O, 2 × C(CH_3)$_3$), 1.22 (s, 3 H; CH_3), 2.08–2.18 (m, 3 H; CH_2(2′A), CH_{2a}(2′B)), 2.26 (ddd, J = 13.2, J = 6.0, J = 3.6 Hz, 1 H; CH_{2b}(2′B)), 2.62 (d, J = 14.4 Hz, 1 H; C(5B)CH_{2a}), 2.82 (d, J = 14.4 Hz, 1 H; C(5B)CH_{2b}), 3.06 (d, J = 13.2 Hz, 1 H; CH_{2a}(6A)), 3.29 (d, J = 13.2 Hz, 1 H; CH_{2b}(6A)), 3.57 (dd, J = 10.0, J = 2.8 Hz, 1 H; CH_{2a}(5′A)), 3.60–3.67 (m, 4 H; 2 × SiCH$_2$CH_2O), 3.72 (dd, J = 12.4, J = 2.4 Hz, 1 H; CH_{2a}(5′B)), 3.76–3.79 (m, 2 H; CH_{2b}(5′A), CH(4′A)),

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3.87–3.89 (m, 1H; CH(4′B)), 3.92 (dd, J = 12.4, J = 2.4 Hz, 1H; CH_{2b}-(5′B)), 4.35–4.38 (m, 1H; CH(3′A)), 4.47–4.50 (m, 1H; CH(3′B)), 5.14 (d, J = 10.0 Hz, 1H; $OCH_{2a}N$(A)), 5.21 (d, J = 9.6 Hz, 1H; $OCH_{2b}N$(A)), 5.34 (d, J = 9.6 Hz, 1H; $OCH_{2a}N$(B)), 5.37 (d, J = 9.6 Hz, 1H; $OCH_{2b}N$(B)), 6.29–6.33 (m, 1H; CH(1′B)), 6.34–6.37 (m, 1H; CH(1′A)), 7.77 ppm (s, 1H; CH(6B)); ^{13}C NMR (100 MHz, $[D_6]$DMSO): δ = −5.3 ($SiCH_3$), −5.2 ($SiCH_3$), −4.7 ($SiCH_3$), −4.5 ($SiCH_3$), −1.3 ($Si(CH_3)_3$), −1.3 ($Si(CH_3)_3$), 18.1 ($C(CH_3)_3$), 18.2 ($SiCH_2CH_2O$), 18.3 ($SiCH_2CH_2O$), 18.5 ($C(CH_3)_3$), 21.6 (CH_3), 25.9 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 32.1 (C(5B)CH_2), 37.1 (CH_2(2′A)), 41.7 (CH_2(2′B)), 43.3 (CH(5A)), 45.2 (CH_2(6A)), 62.0 (CH_2(5′B)), 64.4 (CH_2(5′A)), 67.2 ($SiCH_2CH_2O$(A)), 67.6 ($SiCH_2CH_2O$(B)), 70.3 (OCH_2N(A)), 70.5 (OCH_2N(B)), 72.0 (CH-(3′B)), 73.8 (CH(3′A)), 84.6 (CH(1′A)), 85.0 (CH(4′A)), 86.1 (CH(1′B)), 88.2 (CH(4′B)), 108.5 (C(5B)), 139.2 (CH(6B)), 150.8 (CO(2B)), 152.8 (CO(2A)), 163.8 (CO(4B)), 174.1 ppm (CO(4A)); MS (MALDI$^+$): m/z: 1012 $[M+K^+]$, 996 $[M+Na^+]$; HRMS (MALDI$^+$): m/z calcd for $C_{44}H_{83}N_4NaO_{12}Si_4$: 994.4982; found: 994.4942 $[M+Na]^+$.

Diester 16a: The protected SP model **9a** (10.0 mg, 10.3 µmol), iPr_2NEt (4.30 µL, 25.8 µmol), and 4-pentenoylchloride (2.40 µL, 22.7 µmol) were dissolved in anhydrous CH_2Cl_2 (1 mL) and stirred at RT for 6 h. After 2 h and 4 h stirring, additional iPr_2NEt (4.30 µL, 25.8 µmol) and 4-pentenoylchloride (2.40 µL, 22.7 µmol) were added, respectively. The reaction mixture was diluted with $CHCl_3$ (4 mL), washed with aqueous sodium bicarbonate (5 mL), and the aqueous phase was extracted with $CHCl_3$ (2× 5 mL). The collected organic phases were dried ($MgSO_4$) and the solvent was removed in vacuo. Purification by using flash chromatography (silica gel, i-hexane/ethyl acetate 9:1→1:1) provided **16a** as a colorless oil (11.0 g, 94%).

R_f = 0.73 (iHex/ethyl acetate 7:3); ^{1}H NMR (400 MHz, $[D_4]$MeOH): δ = −0.01 (s, 9H; $Si(CH_3)_3$), 0.00 (s, 9H; $Si(CH_3)_3$), 0.15 (s, 6H; $Si(CH_3)_2$), 0.16 (s, 6H; $Si(CH_3)_2$), 0.85–0.91 (m, 4H; 2×$SiCH_2CH_2O$), 0.94 (s, 9H; $C(CH_3)_3$), 0.96 (s, 9H; $C(CH_3)_3$), 1.23 (s, 3H; C(5A)CH_3), 2.03 (ddd, J = 13.6, J = 5.2, J = 1.2 Hz, 1H; CH_{2a}(2′A)), 2.16–2.24 (m, 2H; CH_{2b}(2′A), CH_{2a}(2′B)), 2.30 (ddd, J = 13.6, J = 6.4, J = 4.0 Hz, 1H; CH_{2b}(2′B)), 2.35–2.42 (m, 4H; 2×CH_2=CHCH_2CH_2), 2.45–2.50 (m, 2H; CH_2=CHCH_2CH_2), 2.54–2.57 (m, 2H; CH_2=CHCH_2CH_2), 2.69 (d, J = 14.0 Hz, 1H; C(5B)CH_{2a}), 2.75 (d, J = 14.0 Hz, 1H; C(5B)CH_{2b}), 3.25 (d, J = 13.2 Hz, 1H; CH_{2a}(6A)), 3.30–3.35 (m, 9H; CH_{2b}(6A), MeOH), 3.58 (t, J = 8.0 Hz, 2H; $SiCH_2CH_2O$(A)), 3.66 (t, J = 8.0 Hz, 2H; $SiCH_2CH_2O$(B)), 3.80 (dd, J = 10.8, J = 3.6 Hz, 1H; CH_{2a}(5′A)), 3.86 (dd, J = 11.2, J = 3.6 Hz, 1H; CH_{2b}(5′A)), 3.93–3.96 (m, 1H; CH(4′A)), 4.04–4.08 (m, 1H; CH(4′B)), 4.27 (dd, J = 12.0, J = 4.0 Hz, 1H; CH_{2a}(5′B)), 4.37 (dd, J = 12.0, J = 5.2 Hz, 1H; CH_{2b}(5′B)), 4.48–4.52 (m, 1H; CH-(3′B)), 4.97–5.02 (m, 2H; CH_2=CH), 5.04–5.06 (m, 1H; CH_2=CH), 5.08–5.10 (m, 1H; CH_2=CH), 5.17 (s, 2H; OCH_2N(A)), 5.22–5.25 (m, 1H; CH(3′A)), 5.34 (s, 2H; OCH_2N(B)), 5.80–5.91 (m, 2H; 2×CH_2=CH), 6.25–6.30 (m, 2H; 2×CH(1′)), 7.62 ppm (s, 1H; CH(6B)); ^{13}C NMR (100 MHz, $[D_4]$MeOH): δ = −5.1 ($SiCH_3$), −5.0 ($SiCH_3$), −4.6 ($SiCH_3$), −4.4 ($SiCH_3$), −1.2 (2×$Si(CH_3)_3$), 18.9 ($C(CH_3)_3$), 19.1 ($SiCH_2CH_2O$(A)), 19.1 ($SiCH_2CH_2O$(B)), 19.4 ($C(CH_3)_3$), 22.4 (C(5A)CH_3), 26.3 ($C(CH_3)_3$), 26.7 ($C(CH_3)_3$), 30.0 (2×CH_2=CHCH_2CH_2), 33.5 (C(5B)CH_2), 34.4 (CH_2=CHCH_2CH_2), 34.5 (CH_2=CHCH_2CH_2), 35.0 (CH_2(2′A)), 41.4 (CH_2(2′B)), 44.2 (C(5A)), 45.8 (CH_2(6A)), 64.5 (CH_2(5′B)), 64.7 (CH_2-(5′A)), 67.8 ($SiCH_2CH_2O$(A)), 68.6 ($SiCH_2CH_2O$(B)), 71.2 (OCH_2N(A)), 71.5 (OCH_2N(B)), 73.3 (CH(3′B)), 75.9 (CH(3′A)), 84.9 (CH(4′A)), 85.7 (CH(1′A)), 86.2 (CH(4′B)), 87.3 (CH(1′B)), 109.9 (C(5B)), 116.2 (2× CH_2=CH), 137.9 (CH_2=CH), 138.0 (CH_2=CH), 140.7 (CH(6B)), 152.1 (CO(2B)), 154.3 (CO(2A)), 165.0 (CO(4B)), 174.1 (ester-CO), 174.3 (ester-CO), 175.3 ppm (CO(4A)); IR (KBr): $\tilde{\nu}_{max}$ = 3080w, 2954s, 2929s, 2897w, 2858m, 1741s, 1719s, 1681s, 1668s, 1463s, 1360m, 1276w, 1249s, 1169w, 1093s, 1030w, 991w, 916m, 860m, 836s, 779m, 694w, 668w, 613w, 521w cm^{-1}; MS (FAB$^+$): m/z (%): 1159 (7) $[M+Na^+]$, 213 (12), 145 (13), 136 (17), 89 (29), 81 (33), 73 (100), 55 (16); HRMS (MALDI$^+$): m/z calcd for $C_{54}H_{96}N_4NaO_{14}Si_4$: 1159.5898; found: 1159.5887 $[M+Na]^+$.

Diester 16b: The reaction was carried out as described above for **16a**. Isomer (R)-**16b** was isolated as a colorless oil (14.0 mg, 80%).

R_f = 0.73 (iHex/ethyl acetate 7:3); ^{1}H NMR (400 MHz, $[D_4]$MeOH): δ = −0.06 (s, 9H; $Si(CH_3)_3$), −0.04 (s, 9H; $Si(CH_3)_3$), 0.09 (s, 6H; $Si(CH_3)_2$), 0.10 (s, 6H; $Si(CH_3)_2$), 0.81–0.87 (m, 4H; 2×$SiCH_2CH_2O$), 0.88 (s, 9H; $C(CH_3)_3$), 0.90 (s, 9H; $C(CH_3)_3$), 1.41 (s, 3H; C(5A)CH_3), 2.00–2.05 (m, 1H; CH_{2a}(2′A)), 2.13–2.22 (m, 2H; CH_{2b}(2′A), CH_{2a}(2′B)), 2.24–2.39 (m, 6H; CH_{2b}(2′B), 2×CH_2=CHCH_2CH_2, C(5B)CH_{2a}), 2.41–2.46 (m, 4H; 2× CH_2=CHCH_2CH_2), 2.87 (d, J = 14.0 Hz, 1H; C(5B)CH_{2b}), 3.20 (d, J = 13.2 Hz, 1H; CH_{2a}(6A)), 3.46 (d, J = 12.8 Hz, 1H; CH_{2b}(6A)), 3.49–3.55 (m, 2H; $SiCH_2CH_2O$(A)), 3.57–3.62 (m, 2H; $SiCH_2CH_2O$(B)), 3.79 (dd, J = 11.2, J = 3.2 Hz, 1H; CH_{2a}(5′A)), 3.83 (dd, J = 11.2, J = 2.8 Hz, 1H; CH_{2b}(5′A)), 3.94–3.96 (m, 1H; CH(4′A)), 4.02–4.06 (m, 1H; CH(4′B)), 4.20 (dd, J = 12.0, J = 4.0 Hz, 1H; CH_{2a}(5′B)), 4.29 (dd, J = 12.0, J = 6.0 Hz, 1H; CH_{2b}(5′B)), 4.38–4.42 (m, 1H; CH(3′B)), 4.91–5.06 (m, 5H; 2×CH_2=CH, $OCH_{2a}N$(A)), 5.14–5.21 (m, 2H; $OCH_{2b}N$(A), CH(3′A)), 5.26 (d, J = 10.0 Hz, 1H; $OCH_{2a}N$(B)), 5.30 (d, J = 10.0 Hz, 1H; $OCH_{2b}N$(B)), 5.74–5.88 (m, 2H; 2×CH_2=CH), 6.16 (t, J = 6.8 Hz, 1H; CH(1′B)), 6.25 (dd, J = 9.6, J = 5.6 Hz, 1H; CH(1′A)), 7.49 ppm (s, 1H; CH(6B)); ^{13}C NMR (150 MHz, $[D_4]$MeOH): δ = −5.2 ($SiCH_3$), −5.0 ($SiCH_3$), −4.7 ($SiCH_3$), −4.5 ($SiCH_3$), −1.2 (2×$Si(CH_3)_3$), 18.9 ($C(CH_3)_3$), 18.9 ($SiCH_2CH_2O$), 19.1 ($SiCH_2CH_2O$), 19.3 ($C(CH_3)_3$), 21.1 (C(5A)CH_3), 26.3 ($C(CH_3)_3$), 26.6 ($C(CH_3)_3$), 29.97 (CH_2=CHCH_2CH_2), 30.00 (CH_2=CHCH_2CH_2), 33.8 (C(5B)CH_2), 34.5 (2×CH_2=CHCH_2CH_2), 35.1 (CH_2(2′A)), 41.5 (CH_2(2′B)), 43.5 (C(5A)), 46.2 (CH_2(6A)), 65.0 (2× CH_2(5′)), 67.8 ($SiCH_2CH_2O$(A)), 68.5 ($SiCH_2CH_2O$(B)), 71.3 (OCH_2N(A)), 71.4 (OCH_2N(B)), 73.9 (CH(3′B)), 76.6 (CH(3′A)), 85.4 (CH(4′A)), 86.1 (CH(1′A)), 86.5 (CH(4′B)), 88.1 (CH(1′B)), 109.8 (C(5B)), 116.2 (2×CH_2=CH), 137.9 (CH_2=CH), 138.0 (CH_2=CH), 139.7 (CH(6B)), 152.1 (CO(2B)), 154.2 (CO(2A)), 164.8 (CO(4B)), 174.1 (ester-CO), 174.2 (ester-CO), 175.1 ppm (CO(4A)); IR (KBr): $\tilde{\nu}_{max}$ = 3080w, 2953s, 2928s, 2856m, 1741s, 1719s, 1670s, 1461s, 1361m, 1276w, 1249s, 1170w, 1092s, 1031w, 992w, 917w, 860m, 835s, 778m, 695w, 669w cm^{-1}; MS (FAB$^+$): m/z (%): 1159 (2) $[M+Na^+]$, 213 (7), 145 (10), 136 (17), 89 (27), 81 (31), 73 (100), 55 (17); HRMS (ESI$^+$): m/z calcd for $C_{54}H_{96}N_4NaO_{14}Si_4$: 1159.5898; found: 1159.5909 $[M+Na]^+$.

Lactone 2a: For the ring-closing metathesis, **16a** (13.0 mg, 11.4 µmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and heated to 40 °C. The *Grubbs*-II catalyst[20,21] (0.50 mg, 0.57 µmol), dissolved in anhydrous CH_2Cl_2 (1 mL), was added and the reaction mixture was stirred at 40 °C for 3 h. The solvent was removed in vacuo. Carrying out flash chromatography (silica gel, i-hexane/ethyl acetate 4:1) gave **2a** as a colorless oil (9.00 mg, 71%).

R_f = 0.25 (iHex/ethyl acetate 4:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = −0.01 (s, 9H; $Si(CH_3)_3$), 0.00 (s, 9H; $Si(CH_3)_3$), 0.03 (s, 3H; $SiCH_3$), 0.04 (s, 3H; $SiCH_3$), 0.10 (s, 3H; $SiCH_3$), 0.11 (s, 3H; $SiCH_3$), 0.86–0.93 (m, 20H; 2×$C(CH_3)_3$, $SiCH_2CH_2O$), 0.96–0.99 (m, 2H; $SiCH_2CH_2O$), 1.24 (s, 3H; C(5A)CH_3), 1.85–1.90 (m, 2H; CH_{2a}(2′A), CH_{2a}(2′B)), 2.12–2.18 (m, 1H; CH_{2b}(2′A)), 2.19–2.24 (m, 1H; 1×CH=CHCH_2CH_2), 2.27 (d, J = 13.8 Hz, 1H; C(5B)CH_{2a}), 2.35–2.49 (m, 8H; 2×CH=CHCH_2CH_2, CH_{2b}(2′B)), 2.68 (d, J = 13.8 Hz, 1H; C(5B)CH_{2b}), 3.24 (d, J = 13.8 Hz, 1H; CH_{2a}(6A)), 3.33 (d, J = 13.8 Hz, 1H; CH_{2b}(6A)), 3.57 (t, J = 8.4 Hz, 2H; $SiCH_2CH_2O$(A)), 3.67 (t, J = 8.4 Hz, 2H; $SiCH_2CH_2O$(B)), 3.74 (dd, J = 11.4, J = 2.4 Hz, 1H; CH_{2a}(5′A)), 3.77 (dd, J = 11.4, J = 2.4 Hz, 1H; CH_{2b}-(5′A)), 4.03–4.06 (m, 1H; CH(4′B)), 4.09–4.12 (m, 1H; CH(4′A)), 4.18–4.21 (m, 2H; CH_{2a}(5′B), CH(3′B)), 4.40 (dd, J = 12.6, J = 3.0 Hz, 1H; CH_{2b}(5′B)), 5.14 (d, J = 9.6 Hz, 1H; $OCH_{2a}N$(A)), 5.18–5.21 (m, 2H; $OCH_{2b}N$(A), CH(3′A)), 5.36 (d, J = 9.6 Hz, 1H; $OCH_{2a}N$(B)), 5.41 (d, J = 9.6 Hz, 1H; $OCH_{2b}N$(B)), 5.44–5.50 (m, 1H; CH=CH), 5.52–5.57 (m, 1H; CH=CH), 6.07 (t, J = 6.0 Hz, 1H; CH(1′B)), 6.37 (dd, J = 10.2, J = 4.8 Hz, 1H; CH(1′A)), 7.09 ppm (s, 1H; CH(6B)); ^{13}C NMR (150 MHz, $[D_4]$MeOH): δ = −5.2 ($SiCH_3$), −5.1 ($SiCH_3$), −4.8 ($SiCH_3$), −4.6 ($SiCH_3$), −1.3 (2×$Si(CH_3)_3$), 18.8 ($C(CH_3)_3$), 18.9 ($SiCH_2CH_2O$), 19.0 ($SiCH_2CH_2O$), 19.3 ($C(CH_3)_3$), 21.0 (C(5A)CH_3), 26.3 ($C(CH_3)_3$), 26.6 ($C(CH_3)_3$), 29.1 (CH=CHCH_2CH_2), 29.7 (CH=CHCH_2CH_2), 33.6 (C(5B)CH_2), 34.4 (CH=CHCH_2CH_2), 34.7 (CH=CHCH_2CH_2), 36.3 (CH_2-(2′A)), 42.6 (CH_2(2′B)), 44.9 (C(5A)), 47.1 (CH_2(6A)), 64.3 (CH_2(5′B)), 64.9 (CH_2(5′A)), 67.9 ($SiCH_2CH_2O$(A)), 68.5 ($SiCH_2CH_2O$(B)), 71.1 (OCH_2N(A)), 71.4 (OCH_2N(B)), 72.9 (CH(3′B)), 76.7 (CH(3′A)), 83.8 (CH(4′A)), 85.5 (CH(1′A)), 87.0 (CH(4′B)), 87.5 (CH(1′B)), 109.8 (C(5B)), 130.8 (CH=CH), 131.0 (CH=CH), 138.4 (CH(6B)), 151.9 (CO(2B)), 154.4 (CO(2A)), 164.9 (CO(4B)), 174.1 (ester-CO), 174.4 (ester-CO), 174.6 ppm (CO(4A)); IR (film): $\tilde{\nu}_{max}$ = 2927s, 2854m, 1737s,

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1667 s, 1461 s, 1360 w, 1249 m, 1089 s, 860 w, 835 s, 778 m cm^{-1}; MS (FAB$^+$): *m/z* (%): 1131 (4) [*M*+Na$^+$], 154 (19), 136 (27), 89 (26), 81 (29), 73 (100); HRMS (ESI$^+$): *m/z* calcd for $C_{52}H_{92}N_4NaO_{14}Si_4$: 1131.5585; found: 1131.5577 [*M*+Na]$^+$.

Lactone 2b: The ring-closing reaction was carried out as described above for **2a**. Isomer (*R*)-**2b** (8.00 mg, 82%) was isolated as a colorless oil.

R_f=0.25 (*i*Hex/ethyl acetate 4:1); ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ= 0.02 (s, 9H; Si(C*H*$_3$)$_3$), 0.03 (s, 9H; Si(C*H*$_3$)$_3$), 0.14 (s, 3H; SiC*H*$_3$), 0.15 (s, 3H; SiC*H*$_3$), 0.17 (s, 6H; Si(C*H*$_3$)$_2$), 0.87–0.91 (m, 4H; 2× SiC*H*$_2$CH$_2$O), 0.95 (s, 18H; 2×C(C*H*$_3$)$_3$), 1.34 (s, 3H; C(5A)C*H*$_3$), 2.09–2.14 (m, 2H; C*H*$_{2a}$(2′A), C*H*$_{2a}$(2′B)), 2.18–2.23 (m, 1H; C*H*$_{2b}$(2′A)), 2.28–2.41 (m, 5H; C*H*$_{2b}$(2′B), 2×CH=CHC*H*$_2$CH$_2$), 2.43–2.50 (m, 4H; 2× CH=CHCH$_2$C*H*$_2$), 2.72 (d, *J*=13.8 Hz, 1H; C(5B)C*H*$_{2a}$), 2.78 (d, *J*= 13.8 Hz, 1H; C(5B)C*H*$_{2b}$), 3.19 (d, *J*=12.6 Hz, 1H; C*H*$_{2a}$(6A)), 3.56–3.60 (m, 2H; SiCH$_2$C*H*$_2$O(A)), 3.64–3.73 (m, 3H; C*H*$_{2b}$(6A), SiCH$_2$C*H*$_2$O(B)), 3.93–3.98 (m, 2H; C*H*$_2$(5′A)), 4.01–4.03 (m, 1H; C*H*-(4′A)), 4.04–4.07 (m, 1H; C*H*(4′B)), 4.12 (dd, *J*=12.0, *J*=6.0 Hz, 1H; C*H*$_{2a}$(5′B)), 4.28 (dd, *J*=12.0, *J*=5.4 Hz, 1H; C*H*$_{2b}$(5′B)), 4.40–4.42 (m, 1H; C*H*(3′B)), 5.03 (d, *J*=9.6 Hz, 1H; OC*H*$_{2a}$N(A)), 5.15 (d, *J*=9.0 Hz, 1H; OC*H*$_{2b}$N(A)), 5.19–5.21 (m, 1H; C*H*(3′A)), 5.36 (s, 2H; OC*H*$_2$N(B)), 5.50–5.60 (m, 2H; C*H*=C*H*), 6.25 (dd, *J*=7.8, *J*=6.0 Hz, 1H; C*H*(1′B)), 6.37 (dd, *J*=10.2, *J*=4.8 Hz, 1H; C*H*(1′A)), 7.44 ppm (s, 1H; C*H*(6B)); ^{13}C NMR (100 MHz, [D$_4$]MeOH): δ=−5.2 (SiCH$_3$), −5.2 (SiCH$_3$), −4.6 (SiCH$_3$), −4.4 (SiCH$_3$), −1.2 (Si(*C*H$_3$)$_3$), −1.2 (Si(*C*H$_3$)$_3$), 18.9 (*C*(CH$_3$)$_3$), 19.0 (Si*C*H$_2$CH$_2$O(A)), 19.1 (Si*C*H$_2$CH$_2$O(B)), 19.3 (*C*-(CH$_3$)$_3$), 22.3 (C(5A)*C*H$_3$), 26.3 (C(*C*H$_3$)$_3$), 26.6 (C(*C*H$_3$)$_3$), 28.6 (CH=CH*C*H$_2$CH$_2$), 28.7 (CH=CH*C*H$_2$CH$_2$), 34.3 (CH=CHCH$_2$*C*H$_2$), 34.4 (*C*H$_2$(2′A)), 34.7 (C(5B)*C*H$_2$), 34.8 (CH=CHCH$_2$*C*H$_2$), 41.2 (*C*H$_2$(2′B)), 43.3 (*C*(5A)), 46.8 (*C*H$_2$(6A)), 64.7 (*C*H$_2$(5′B)), 65.2 (*C*H$_2$(5′A)), 67.9 (SiCH$_2$*C*H$_2$O(A)), 68.6 (SiCH$_2$*C*H$_2$O(B)), 71.2 (O*C*H$_2$N(A)), 71.4 (O*C*H$_2$N(B)), 74.0 (*C*H(3′B)), 77.5 (*C*H(3′A)), 86.2 (*C*H(1′A)), 86.4 (2× *C*H(4′)), 87.3 (*C*H(1′B)), 111.1 (*C*(5B)), 130.7 (CH=CH), 131.3 (CH=*C*H), 138.7 (*C*H(6B)), 152.2 (*C*O(2B)), 154.4 (*C*O(2A)), 164.6 (*C*O(4B)), 174.2 (ester-*C*O), 174.4 (ester-*C*O), 174.6 ppm (*C*O(4A)); IR (film): $\tilde{\nu}_{max}$=2953 s, 2929 s, 2857 m, 1732 s, 1682 s, 1461 m, 1361 w, 1248 s, 1080 s, 936 w, 860 w, 835 s, 778 m cm^{-1}; MS (FAB$^+$): *m/z* (%): 1131 (5) [*M*+Na$^+$], 154 (19), 136 (28), 89 (30), 81 (30), 73 (100); HRMS (ESI$^+$): *m/z* calcd for $C_{52}H_{92}N_4NaO_{14}Si_4$: 1131.5585; found: 1131.5568 [*M*+Na]$^+$.

Enzymatic-repair assay: SplG was overexpressed and purified as His$_6$-tagged recombinant protein in *Escherichia coli*.[42] The reconstitution of the {Fe-S} cluster and the repair assays were performed under anaerobic conditions in a glove box. For enzyme activity, the amount of 5′-deoxyadenosine and repaired SP was determined and analyzed by using rp-HPLC. Each reaction mixture contained Tris-HCl (pH 7.0, 100 mM), KCl (200 mM), sodium dithionite (3 mM), DTT (5 mM), and SAM (0.45 mM). All solutions were degassed under reflux and the chemicals were transferred into the glove box as solids. One sample only contained the components described above. To the second sample 50 µmol holo-splG was added. The third sample contained 50 µmol splG and the 5*S*-configured spore lesion. The fourth one contained the same as the third, but instead of the 5*S*-configured spore lesion the 5*R*-configured spore lesion was added. All the reaction mixtures were incubated for 12 h at 4°C for comparison. After incubation, the reaction mixture was frozen in liquid nitrogen and stored at −80°C. All the samples were thawed and centrifuged to remove precipitated protein before being injected into the HPLC column.

rp-HPLC analysis: For the rp-HPLC analysis, the samples were injected directly after they had been centrifuged in order to decrease the SAM cleavage to *S*-adenosylhomocysteine. Up to 50 µL of the enzyme reaction were injected onto the rp-HPLC column (Macherey–Nagel Nucleosil 300-5 C18) equilibrated with 0.1% TFA in H_2O. The products could be separated by applying a linear gradient (0%→30% solvent B (50% CH_3CN with 0.1% TFA) in 20 min) at a flow rate of 0.7 mL min^{-1}. The detection wavelength was 260 nm. The HPLC gradient was chosen to allow separation and hence detection of thymidine in the assay solution, which is the only expected product of the repair reaction. The peaks were assigned by coinjection of thymidine and 5′-deoxyadenosine, and were further analyzed by Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (Thermo Finnigan LTQ FT) measurements.

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