HHS Public Access

Author manuscript

Eur J Med Chem. Author manuscript; available in PMC 2018 February 15.

Published in final edited form as:

Eur J Med Chem. 2017 February 15; 127: 793–809. doi:10.1016/j.ejmech.2016.10.059.

Oligonucleotide transition state analogues of saporin L3

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Abstract

Ribosome inactivating proteins (RIPs) are among the most toxic agents known. More than a dozen clinical trials against refractory cancers have been initiated using modified RIPs with impressive results. However, dose-limiting toxicity due to vascular leak syndrome limits success of the therapy. We have previously reported some tight-binding transition state analogues of Saporin L3 that mimic small oligonucleotide substrates in which the susceptible adenosine has been replaced by a 9-deazaadenyl hydroxypyrrolidinol derivative. They provide the first step in the development of rescue agents to prevent Saporin L3 toxicity on non-targeted cells. Here we report the synthesis, using solution phase chemistry, of these and a larger group of transition state analogues. They were tested for inhibition against Saporin L3 giving K_i values as low as 3.3 nM and indicating the structural requirements for inhibition.

Keywords

Saporin; Ribosome inactivating protein; Transition state inhibitor; Oligonucleotide; Aza-sugar

1. Introduction

Saporins are ribosome inactivating proteins (RIPs) isolated from the soapwort (*Saponaria officinalus*) [1]. Like the better known ricin A-chain (RTA), saporins bind to the sarcin – ricin loop of the 28S eukaryotic ribosomal RNA and hydrolytically depurinate adenosine 4234 [2], thereby preventing protein synthesis and causing cell death. Saporins are less specific than RTA, as they also hydrolyze adenine from other sites on RNAs.

Trial cancer therapies have exploited the toxicity of saporin and RTA – antibody constructs targeted to leukemia and lymphoma cells [3]. Off-target toxicity resulting from incomplete uptake or release of RIPs from dying target cells limits the use of these therapies. Inhibitors of RIPs could provide therapy enhancement by inhibition of unwanted RIP following the initial treatment [4]. This paper describes the synthesis and characterization of several potent

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Supporting information

NMR spectra of all compounds. HPLC traces for compounds **2**, **3**, **23**, **25**, **30**, **32**, **33**, **39**, **41**, **45** and **52**.

inhibitors of saporin L3 (SAP) [5,6] a highly active saporin isoform originally isolated from *S. officinalus* leaves. The inhibitors are characterized with saporin L3 expressed in yeast and mutated to replace alanine 14 with cysteine (SAP A14C), a mutation remote from the catalytic site which provides a chemical attachment site for cell-recognition molecules.

The transition states for RTA and SAP-catalyzed depurination of RNAs have ribocation character from which activated adenine is largely dissociated [7]. The nonhydrolysable adenosine mimic **1** (DIA) captures, in stable form, key features of the transition state geometry. The extended bond length between leaving group adenine and ribose is mimicked by the methylene bridge, the imino-sugar is protonated at physiological pH to mimic the ribocation, and N7 of the 9-deazaadenine ring is protonated at physiological pH. DIA does not significantly inhibit either SAP or RTA, but replacement of the susceptible adenosine in RNA sequences from the reactive part of the sarcin – ricin loop with DIA gives potent inhibitors [8]. The stem loop A-10 (5′-CGCGAGAGCG-3′) along with linear and cyclized GAGA constructs are accepted as substrates by SAP [4], and these molecules provide a template for inhibitor design. SAP accepts truncated RNAs as substrates and is inhibited by the corresponding aza-sugar constructs under physiological conditions whereas RTA requires reduced pH for activity on truncated substrates and for inhibition [4,8].

The crystal structure of a cyclic G(DIA)GA tetramer bound to SAP shows a quadruple **π**-stack in which deazaadenine is positioned between two tyrosine phenol groups with the 3′-guanosine providing the final layer of the **π**-stack. These interactions, together with multiple hydrogen bonds, are proposed to provide leaving group activation in catalytic structures [9].

The RNA oligonucleotides containing DIA, **2**–**4** (Fig. 1), are potent inhibitors of SAP [4]. These oligonucleotides are stabilised against ribonucleases by 2′-O-methylation [10] which, in the context of inhibitor design, is tolerated by SAP [11] and some have phosphate end groups capped with propanediol. They were synthesized in the usual manner for oligonucleotides, on solid support with stepwise addition of each nucleotide phosphoramidite, often requiring multiequivalents of each. We have found that aza -sugar phosphoramidites such as **14** (Scheme 1) give poor and non-reproducible yields under this protocol.

Using conventional solution phase chemistry, which allows for convergent strategies and more efficient use of valuable intermediates, we have synthesized a group of aza-sugar-containing 2′-O-methyl di, tri and tetra RNA nucleotides that probe both aza-sugar structure and the nucleotide context required for inhibition of SAP [12,13]. Compounds 2–4 were resynthesised for comparison and to provide sufficient material for chemical characterization.

2. Results and discussion

2.1. Chemistry

Tetramer **4** (G*p*DIA*p*G*p*A) is an inhibitor construct corresponding to the GAGA substrate of SAP. It was synthesized in a linear fashion by stepwise addition to the 3′-terminal adenosine (Scheme 1). Reaction of the 3′-hydroxyl of commercially available 5′-*O*-dimethoxytrityl-6-

N-benzoyl-2′-*O*-methyladenosine **6** with chloroacetyl chloride followed by hydrolysis of the dimethoxytrityl (DMTr) group with aqueous acetic acid gave 5′-OH adenosine **7** in good yield. The phosphate linkages were protected as benzyl esters as they provide the additional stability over the cyanoethyl group required for solution phase work. Benzyloxydiamidite **5**, synthesized from phosphorus trichloride in one pot and purified by partition between hexane and acetonitrile [14], was coupled with guanosine **8** to give the amidite building block **9** which was coupled with adenosine **7** using 5-methyltetrazole (MTET) as the activator. To the best of our knowledge, MTET has not previously been used as an activator in phosphoramidite coupling reactions. It's predicted pK_a of 5.1 [15] is close to that of the commonly used 4,5 – dicyanoimidazole (DCI, pK_a 5.0) and it is not subject to the same shipping restrictions as tetrazole. It was effective in many of the reactions reported herein. Oxidation of the intermediate phosphite ester with aqueous 70% *t*-butylhydroperoxide afforded the benzyl protected phosphate ester **10** in excellent yield as a mixture of diastereomers at phosphorus. After purification by silica gel chromatography the DMTr group was removed with acetic acid to give **11** in good yield.

A Mannich reaction [16] between 6-*N*-benzoyl-9-deazaadenine, (3*R*, 4*R*)-4-(hydroxymethyl)-pyrrolidin-3-ol [17] and formaldehyde gave benzoyl-protected DIA **12** which was selectively tritylated on the primary hydroxyl in good yield to give **13**. Preparation of amidite **14** was initially problematic as it was readily hydrolysed (to an H-phosphonate) during purification on silica gel. However, optimization of the chromatography to give rapid elution of **14** as well as resolution from impurities resulted in good yields. Once purified, **14** was stable for at least a week at room temperature and for months at −20 °C. It was coupled with dimer **11** followed by hydrolytic removal of DMTr to give **15** in reasonable yield. Further extension with guanosine phosphoramidite **9** and detritylation gave **16** in poor yield which was fully deprotected, first by hydrogenolysis followed by heating in ammonium hydroxide to give the target **4**.

Synthesis of constructs bearing a DIAeguanosine-3′epropanediol diphosphate (DIA*p*G*p*PD) is illustrated in Scheme 2. Coupling of propanediol amidite **17** and guanosine **8** followed by oxidation of the intermediate phosphite ester gave **18** in good yield. Removal of the DMTr group with methanolic HCl [12] gave **19** in quantitive yield. Instead of using DIA amidite **14** to extend synthon **19** we investigated an alternate approach in which **19** was converted to amidite **20** and then reacted with DIA alcohol **13**. Coupling of **20** with **13** catalyzed by tetrazole, gave a moderate yield of **21**. Removal of the DMTr group from **21** gave alcohol **22** which was utilized in several ways. Global deprotection gave **23** (DIA*p*G*p*PD), reaction with propanediol amidite **17** followed by deprotection gave **2** (PD*p*DIA*p*G*p*PD), reaction with guanosine amidite **7** and deprotection gave **3** (G*p*DIA*p*G*p*PD), and further extension of **24** gave **25** (PD*p*G*p*DIA*p*G*p*PD).

Compounds **30** (DIA*p*G), **31** (G*p*DIA*p*G) and **33** (PD*p*DIA*p*G) were synthesized in a stepwise fashion from either 3′-*O*-benzoyl or –elevulinoyl guanosines **26** and **27** (Scheme 3).

A convergent synthesis of **39** (PD*p*G*p*DIA*p*PD) from protected PD*p*G and DIA*p*PD synthons is illustrated in Scheme 4. The orthogonal protection afforded by the levulinoyl

protective group was used in the preparation of PD*p*G amidite synthon **36**. Thus, 5′-hydroxy levulinoyl guanosine **27** was coupled with propanediol amidite **17** and oxidized to **34**. Hydrazine effected selective removal of the levulinoyl group in excellent yield and the resulting alcohol **35** was converted to phosphoramidite **36**. Reaction with synthon **37** (prepared from propanediol amidite **17** and DIA alcohol **13)** gave a moderate yield of **38** and standard deprotection protocol gave **39**. We attempted an analogous convergent strategy to synthesize **41** but coupling of the required diguanosine phosphoramidite with **37** was not successful. Compound **41** was better prepared in a stepwise fashion by two additions of guanosine phosphoramidite **9** followed by deprotection (Scheme 4).

The methylene bridge between the pyrrolidine and deazaadenine in DIA models the dissociative nature of the transition state for hydrolysis by SAP. In compound **42** (DEIA) we have increased the pyrrolidine – deaazaadenine distance by incorporation of an ethylene bridge as a comparison. Mesylation of 9-hydroxyethyl-9-deazaadenine [18] and nucleophilic displacement with (3*R*, 4*R*)-4-(hydroxymethyl)-pyrrolidin-3-ol [17] gave **42** (Scheme 5) which was selectively tritylated to give **43**. The potentially troublesome conversion of **43** to a phosphoramidite was circumvented by treating **43** with the PD*p*G amidite **20** followed by hydrolysis of the DMTr group to give **44**. Compound **45** (PD*p*DEIA*p*G*p*PD) was then accessed by the deprotection methods described above.

Constructs with serinol as the aza-sugar component are potent inhibitors of SAP [19]. The use of homoserinol (2-(aminomethyl) propane-1,3-diol) in this context offers the possibility for additional flexibility enabling stronger interactions with SAP. The Mannich reaction between protected homoserinol **46**, formadehyde and 9-deazaadenine gave **47** (Scheme 6) [20,21] Standard protecting group manipulations gave racemic monotrityl compound **49** which was treated with phosphorodiamidite **5** and then the product was coupled with guanosine derivative **19** to give **50**. Acid hydrolysis gave **51** and then coupling with amidite **17** followed by global deprotection gave racemic **52**. NMR peaks of Fmoc protected compounds **49**–**51** were extremely broad, presumably due to NMR-timescale inversion or rotation whereas **52** had well resolved NMR spectra consistent with the desired structure.

2.2. Inhibitory studies

Inhibition of SAP was studied in a competitive assay using stem-loop RNA A10 (5′CGCGAGAGCG3′) as the substrate. Product formation was quantitated in continuous assays by linking formation of adenine from RNA substrates to the production of light from luciferase [4].

The DIA (**1**) inhibitor constructs show strong relationships between structure and activity. All constructs with dissociation constants (K_i* values, including slow-onset properties) < 40 nM contain the DIA*p*G substructure. When complexed with SAP this moiety forms a quadruple **π**-stack with the phenolic rings of SAP tyrosines 73 and 123 [9]. Our data shows this stack is essential but not sufficient for binding. Comparison of compounds **23** and **30** with **2**, **3**, **4** and **25** indicates that tight binding requires the DIA*p*G motif to be flanked by phosphate esters, i.e. ***p***DIA*p*G***p***. Hydrogen bonds to these terminal phosphates are evident in inhibitor – SAP crystal structures and are important for stabilization of the SAP – nucleotide

complex [9]. Compounds **39** and **41** which have three phosphate groups but not the DIA*p*G substructure are weak inhibitors. Propanediol can replace the terminal G and A of the inhibitor **6**; either separately (**3**) or together (**2**) with little effect on inhibition. Adding an additional phosphate ester to **3**, as in **25**, does not increase inhibitor affinity. A crystal structure of a circular construct of *p*G*p*DIA*p*G*p*A*p* complexed with SAP shows interactions with SAP only through the central *p*DIA*p*G*p* motif [9] and the correlation between structure and inhibition presented here are fully consistent with this binding model.

The PD*p*DEIA*p*G*p*PD construct **45** with an additional methylene separating the aza-sugar and deazaadenine is almost as good an inhibitor as the equivalent DIA analogue **2**, indicating that SAP can accommodate both increased distance from the phosphodiester backbone and additional flexibility. Constructs containing acylic ribocation mimics homoserinol (**52**) and serinol [19] are also good inhibitors, but less potent than those containing the cyclic amines.

The molecular electrostatic potential surfaces (MEPs) of compounds **2**, **45** and **52** reveal similarity to the MEP of the transition state (Fig. 2) [22] while the MEP of the catalytically inactive substrate analogue PD*p*A*p*GPD differs significantly, which is also consistent with the weaker binding of this compound (K_i* = 16.1 μM; Fig. 2 and Table 1).

Compound **2** also inhibits the hydrolysis of yeast tRNA and rabbit rRNA by both SAP and SAP-A14C (Fig. 3). In rabbit reticulocyte cell-free protein translation studies, SAP A14C inhibited translation of luciferase mRNA with an IC_{50} of 330 pM. Addition of inhibitor **2** to translation mixtures rescued the translation of luciferase with an IC_{50} of 13 nM in the presence of 10 nM SAP A14C (Fig. 4). Thus, a stoichiometric excess of 3 nM of compound **2** relative to SAP is required for rescue of translation, fully consistent with the K_i* value of 3.3 nM (Table 1).

3. Conclusion

We have synthesized a suite of oligonucleotide inhibitors of SAP using solution phase techniques. These compounds define the structural parameters required for inhibition of SAP that is consistent with x-ray crystal structure studies. The low nanomolar dissociation constants for the best compounds encourage physiological applications as SAP rescue agents.

4. Experimental section

4.1. General

Air sensitive reactions were performed under argon. Organic solutions were dried over anhydrous $MgSO_4$ and the solvents were evaporated under reduced pressure. Anhydrous and chromatography solvents were obtained commercially and used without any further purification. Thin layer chromatography (tlc) was performed on aluminium sheets coated with 60 F_{254} silica gel. Organic compounds were visualized under uv light or using a dip of *N*,*N*-dimethylaminobenzaldehyde (0.6%) in methanol – conc. HCl, 6:1, or $KMnO_4$ (0.5% in water). Column chromatography was performed on silica gel (40–63 μm) or on prepacked columns using an automated system with continuous gradient facility. ^{1}H NMR spectra were

measured in $CDCl_3$, CD_3OD (internal Me_4Si, δ 0) or D_2O (HOD, δ 4.79). ^1H-decoupled ^{13}C NMR spectra were measured in $CDCl_3$ (centre line, δ 77.0), CD_3OD (centre line, δ 49.0) or D_2O (no internal reference). ^1H-decoupled ^{31}P NMR spectra were measured with no internal reference. Assignments of ^1H and ^{13}C resonances were based on 2D (^1H-^1H DQF-COSY, ^1H-^{13}C HSQC, HMBC, ^{31}P-^1H COSY) and DEPT experiments. HPLC used either a Kinetex C-18 column eluted with a gradient of MeCN in water containing 0.1% TFA or a Poroshell 120 EC-C-18 column eluted with a gradient of MeOH in water containing 0.01% formic acid or Et_3NHOAc (10 mM, pH 6.5). Detection was at 254 nM. LCMS utilized a quadrupole mass spectrometer with electrospray ionization. Preparative HPLC utilized a Luna C-18 column eluted with *ca* 20% MeOH in Et_3NHOAc (50 μM, pH 6.5) High resolution mass spectra (HRMS) were recorded with electrospray ionization on a Q-TOF tandem mass spectrometer.

5. Methods

5.1. Method 1 – coupling and oxidation

Alcohol (1 equiv) and phosphoramidite (1.5–4 equiv) were dissolved in a little dry DCM and diluted with dry acetonitrile. The solution was concentrated and held under oil pump vacuum for 1 h. Dry DCM (approx. 20 mL/g) and activator (tetrazole, MTET or 4, 5-dicyanoimidazole (DCI), 2–5 equiv) were added and the solution stirred for 1–2 h when tlc or HPLC showed conversion of the alcohol to a new material. *t*-Butylhydroperoxide (20% solution in toluene or 70% solution in water, 3–6 equiv) was added and the solution stirred for a further 3 h. The solution was diluted with $CHCl_3$ and extracted with $Na_2S_2O_3$ (10%, aq), $NaHCO_3$ (10%, aq) and brine. Drying, concentration and chromatography on silica, eluting with a gradient (0–15%) of MeOH in $CHCl_3$ – EtOAc, 2:1 (unless otherwise stated), gave the phosphate ester product as a mixture of diastereomers.

5.2. Method 2 – hydrolytic cleavage of the dimethoxytrityl group

Methanolic HCl (5 μM) was freshly prepared from MeOH and AcCl. DMTr nucleotide (10–20 mg/mL) was stirred in this for 10–20 min, when tlc showed complete reaction. The solution was bought to pH 7 by the addition of $NaHCO_3$ (aq, 10%) and concentrated to dryness. The residue was taken up in $CHCl_3$, $MgSO_4$ was added and the solution filtered through diatomaceous earth. The detritylated nucleotide was isolated by chromatography, eluting with a gradient (0–15%) of MeOH in $CHCl_3$ – EtOAc, 2:1.

5.3. Method 3 – global deprotection of oligonucleotides

The protected oligonucleotide (5–100 mg) was dissolved in THF (5–20 mL) and stirred with $Pd(OH)_2$ on carbon (50 wt%) or Pd on carbon (5%) or Pd black under a balloon of hydrogen. After 24 h, or when LCMS showed hydrogenolysis of all the benzyl groups, water (5 mL) was added and the catalyst removed by filtration. Solvents were removed and the residue taken up in ammonium hydroxide (27%). The solution was stirred at 50 °C for 24 h and then concentrated and purified as described.

5.4. Compound 7

To a solution of **6** (1.18 g, 1.72 mmol) in dry CH_2Cl_2 (10 mL) was added pyridine (0.421 mL 5.15 mmol) and then chloroacetyl chloride (0.195 mL, 2.40 mmol) and the resulting solution was stirred at rt for 1 h, then washed with water, HCl (1 M), $NaHCO_3$ (10%, aq), dried and concentrated to dryness. Chromatography (40% and 50% EtOAc in $CHCl_3$ gave a foam (1.07 g, HRMS – found 764.2476, calc for $C_{41}H_{39}N_5O_8Cl$ $[M+H]^+$, 764.2487) which was dissolved in AcOH (80%, aq, 20 mL) and stirred at rt for 2 h. Concentration and chromatography (EtOAc) gave **7** (0.54 g, 1.1 mmol, 71%) as a colourless foam. ^1H NMR ($CDCl_3$, 500 MHz) δ 9.26 (s, 1H), 8.77 (s, 1H), 8.10 (s, 1H), 8.06–7.99 (m, 2H), 7.65–7.58 (m, 1H), 7.52 (dd, J = 10.6, 4.8 Hz, 2H), 6.18 (d, J = 10.9 Hz, 1H), 5.90 (d, J = 7.9 Hz, 1H), 5.75 (d, J = 4.9 Hz, 1H), 4.82 (dd, J = 7.9, 4.9 Hz, 1H), 4.40 (s, 1H), 4.24–4.17 (m, 2H), 4.00 (d, J = 13.0 Hz, 1H), 3.83 (t, J = 11.8 Hz, 1H), 3.29 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.5, 164.6, 152.2, 150.6, 150.5, 143.2, 133.4, 133.0, 128.9, 128.0, 124.7, 89.4, 85.8, 81.2, 74.0, 62.8, 59.6, 40.6. HRMS – found 462.1176, calc for $C_{20}H_{21}N_5O_6Cl$ $[M+H]^+$ 462.1180.

5.5. Compound 9

A solution of **8** (3.0 g, 4.5 mmol) and MTET (0.33 g, 3.9 mmol, 0.88 equiv) in dry CH_2Cl_2 (100 mL) was added to a solution of **5** (3.0 g, 9.0 mmol, 2.0 equiv) in dry CH_2Cl_2 (30 mL). After stirring for 2 h, Et_3N (1.3 mL) was added and the solution was concentrated to a small volume. Chromatography eluting with hexanes – $CHCl_3$ 1:1, $CHCl_3$ and then 3% MeOH in $CHCl_3$, all containing 1% Et_3N gave **9** (3.92 g, 4.3 mmol, 96%) as a 3:2 mix of stereoisomer. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78 (m, 1H), 7.54 (m, 2H), 7.40 (m, 4H), 7.34–7.08 (m, 10H), 6.78 (m, 4H), 5.84 (t, J = 7.4 Hz, 1H), 4.76 (m, 2H), 4.64 (m, 1H), 4.49 (m, 1H), 4.31 (m, 1H), 3.74 (m, 6H), 3.62 (m, 2H), 3.51 (m, 1H), 3.43 (m, 3H), 3.11 (m, 1H), 2.54 (q, J = 7.3 Hz, 1H), 1.22–0.71 (m, 18H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 178.3, 158.8, 155.7, 148.3, 147.1, 145.1, 144.9, 138.8, 136.1, 135.7, 130.0, 128.2, 128.1, 128.0, 127.3, 127.1, 127.0, 126.9, 122.5, 113.3, 86.6, 86.4, 84.5, 84.1, 81.8, 81.2, (71.0, 70.1) both d, $J_{C,P}$ = 17 Hz), (65.8, 65.2, both d, $J_{C,P}$ = 18 Hz), 63.3, 58.7, 58.2, 55.2, 46.2, (43.3, 43.0, both d, $J_{C,P}$ = 13 Hz), 36.0, 24.7, 24.6, 24.5, 24.4, 18.5. ^{31}P NMR ($CDCl_3$, 202 MHz)δ 150.7, 149.9.

5.6. Compound 10

7 (1.24 g, 2.68 mmol) and phosphoramidite **9** (3.70 g, 4.08 mmol) were coupled according to the Method 1 using MTET (1.5 equiv) as the activator. Chromatography (0–10% MeOH in EtOAc) gave **10** (2.95 g, 2.30 mmol, 85%), a foam, as a mixture of stereoisomers. ^1H NMR ($CDCl_3$, 500 MHz) δ 11.98 and 11.93 (s, 1H), 9.13 (m, 2H), 8.80 and 8.75 (s, 1H), 8.33 and 8.18 (s, 1H), 8.05–7.93 (m, 2H), 7.80 and 7.76 (s, 1H), 7.63–7.44 (m, 3H), 7.43–7.10 (m, 15H), 6.80–6.70 (m, 4H), 6.15 and 6.06 (d, J = 5.6 Hz, 1H), 5.83 and 5.78 (d, J = 6 Hz, 1H), 5.69–5.48 (m, 2H), 5.17–5.08 (m, 1H), 4.99–4.74 (m, 2H), 4.48–4.07 (m, 4H), 3.74, 3.73, 3.73 and 3.72 (s, 2 × 3H), 3.48, 3.39, 3.35 and 3.32 (s, 2 × 3H), 3.47–3.28 (m, 1H), 3.19 and 3.07 (dd, J = 11.0, 3.0 Hz, 1H), 2.16–2.01 (m, 1H), 1.03, 0.97, 0.92 and 0.86 (d, J = 6.9 Hz, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.0,178.8, 166.6, 166.5, 164.8, 164.7, 158.7, 155.5, 155.4, 152.9, 152.8, 151.8, 151.6, 149.8, 148.1, 147.7, 147.6, 144.5, 142.1,

141.8, 138.7, 138.4, 135.6, 135.3, 135.0, 133.2, 133.0, 130.0, 129.2, 128.9, 128.8, 128.7, 128.0, 128.0, 127.8, 127.2, 127.0, 123.9, 122.0, 113.3, 113.2, 87.2, 87.1, 86.6, 85.4, 82.1, 81.9, 81.2, 81.1, 80.8, 80.7, 80.6, 75.1, 72.3, 72.2, 70.2, 70.0, 66.3, 66.1, 62.5, 60.4, 59.4, 58.8, 58.7, 55.2, 40.4, 36.13, 36.08, 18.72, 18.66, 18.57. HRMS – found 1283.3995, calc for $C_{63}H_{65}ClN_{10}O_{16}P$ $[M+H]^+$, 1283.4006.

5.7. Compound 11

Compound **10** (2.80 g, 2.18 mmol) in AcOH (80% aq, 30 mL) was stirred at rt. After 3 h, $CHCl_3$ was added and the mixture was washed with water (×2) and then $NaHCO_3$ (10%, aq), dried and evaporated. Chromatography (0–20% MeOH in EtOAc) gave two stereoisomers of **11** as colourless foams, first isomer A, (0.67 g) and then isomer B (0.89 g, total 1.56 g, 1.58 mmol, 72%). For Isomer A: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.21 (s, 1H), 10.19 (s, 1H), 9.50 (s, 1H), 8.80 (s, 1H), 8.35 (s, 1H), 8.11–7.91 (m, 3H), 7.64–7.51 (m, 1H), 7.51–7.41 (m, 2H), 7.40–7.22 (m, 5H), 6.14 (d, *J* = 5.7 Hz, 1H), 5.78 (t, *J* = 7.2 Hz, 1H), 5.60 (dd, *J* = 5.1, 3.6 Hz, 1H), 5.23–5.04 (m, 4H), 4.88 (t, *J* = 5.5 Hz, 1H), 4.54 (t, *J* = 5.3 Hz, 1H), 4.46–4.36 (m, 3H), 4.18 (s, 2H), 3.88–3.80 (m, 1H), 3.58 (d, *J* = 11.9 Hz, 1H), 3.37 (s, 3H), 3.28 (s, 3H), 2.88 (s, 1H), 2.81–2.71 (m, 1H), 1.19–1.14 (m, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.8, 166.7, 165.1, 155.4, 152.8, 151.8, 149.9, 148.2, 148.0, 142.2, 138.9, 135.2, 135.1, 133.2, 132.9, 129.0, 128.84, 128.78,128.1, 128.0, 123.9, 121.7, 87.1, 86.8, 84.9, 81.7, 80.9, 80.8, 76.1, 72.3, 70.2, 70.1, 66.4, 61.5, 59.4, 58.7, 40.5, 36.1, 19.1, 18.8. HRMS-found 981.2706, calc for $C_{42}H_{47}ClN_{10}O_{14}P$ $[M+H]^+$, 981.2699. For isomer B: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.13 (s, 1H), 10.19 (s, 1H), 9.51 (s, 1H), 8.75 (s, 1H), 8.33 (s, 1H), 8.10–7.86 (m, 3H), 7.54 (t, *J* = 7.4 Hz, 1H), 7.50–7.22 (m, 7H), 6.11 (d, *J* = 5.6 Hz, 1H), 5.74 (d, *J* = 6.1 Hz, 1H), 5.60 (dd, *J* = 4.9, 3.9 Hz, 1H), 5.33 – 5.04 (m, 4H), 4.87 (t, *J* = 5.4 Hz, 1H), 4.55 (t, *J* = 5.1 Hz, 1H), 4.45–4.19 (m, 3H), 4.18 (s, 2H), 3.88 (d, *J* = 10.1 Hz, 1H), 3.67 (d, *J* = 11.8 Hz, 1H), 3.36 (s, 3H), 3.27 (s, 3H), 2.94 (s, 1H), 2.78–2.69 (m, 1H), 1.18 (m, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.6, 166.7, 165.1, 155.5, 152.7, 151.8, 149.9, 148.1, 147.9, 142.3, 138.8, 135.23, 135.18, 133.2, 132.9, 129.0, 128.8, 128.1, 127.9, 124.0, 121.5, 87.1, 86.8, 84.4, 81.5, 80.7, 75.6, 72.2, 70.3, 70.2, 66.2, 61.1, 59.4, 58.6, 40.5, 36.1, 18.9. HRMS Found 981.2704, calc for $C_{42}H_{47}ClN_{10}O_{14}P$ $[M+H]^+$, 981.2699.

5.8. Compound 12

Aqueous formaldehyde (1.25 mL, 16.6 mmol, 37 wt %) was added to a suspension of (3*R*, 4*R*)-4-(hydroxymethyl)-pyrrolidin-3-ol (1.95 g, 16.6 mmol) [17] and 6-*N*-benzoyl-9-deazaadenine (3.6 g, 15.1 mmol) in H_2O (40 mL) and EtOH (20 mL) and the mixture warmed to 60°C. After 4 h tlc analysis of the homogeneous reaction mixture showed none of the starting amine. Silica gel (ca 20 g) was added and the resulting suspension concentrated to afford a solid residue which was chased consecutively with MeOH and $CHCl_3$. The resulting residue was purified by chromatography on silica gel (20%–40% MeOH in $CHCl_3$ then 1:1 dioxane:H_2O) to afford **12** (5.55 g, 52%) as a pale yellow solid. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.57 (s, 1H), 8.10 (m, 2H), 7.75 (s, 1H), 7.64 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.57–7.53 (m, 2H), 4.00 (dt, *J* = 6.2, 4.1 Hz, 1H), 3.93 (d, *J* = 13.4 Hz, 1H), 3.89 (d, *J* = 13.4, 1H), 3.62 (dd, *J* = 10.7, 5.9 Hz, 1H), 3.50 (dd, *J* = 10.8, 7.7 Hz, 1H), 3.00 (dd, *J* = 9.8, 8.3 Hz, 1H), 2.84 (dd, *J* = 10.1, 6.3 Hz, 1H), 2.67 (dd, *J* = 10.1, 4.2 Hz, 1H), 2.44 (dd, *J* = 9.8, 6.7

Hz, 1H), 2.21–2.14 (m, 1H). ^{13}C NMR (CD_3OD, 126 MHz) δ 168.8, 151.7, 150.1, 144.7, 134.5, 133.9, 133.1, 129.8, 129.4, 117.9, 112.4, 74.2, 64.2, 62.6, 56.8, 51.2, 48.9. HRMS found, 368.1724 calc for $C_{19}H_{22}N_5O_3$ $[M+H]^+$, 368.1723.

5.9. Compound 13

12 (2.90 g, 7.893 mmol) was dissolved in pyridine (50 mL), concentrated under vacuum and then redissolved in pyridine (65 mL). 4,4′-DMTrCl (3.03 g, 8.68 mmol) was added and the resulting mixture stirred for 1 h at rt under an argon atmosphere. The reaction mixture was diluted with $CHCl_3$ (250 mL), washed with H_2O (100 mL) and then $NaHCO_3$ (10%, aq.), and the organic layer was dried, filtered and concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$ then EtOAc, then 10%–20% MeOH in $CHCl_3$) to afford **13** (4.45 g, 84%) as a syrup.^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.93 (s, 1H), 9.40 (s, 1H), 8.49 (s, 1H), 8.01–7.99 (m, 2H), 7.61 (tt, *J* = 7.4, 1.8 Hz, 1H), 7.52–7.49 (m, 3H), 7.40–7.38 (m, 2H), 7.29–7.23 (m, 7H), 7.18 (tt, *J* = 7.3, 2.0 Hz, 1H), 6.81–6.78 (m, 4H), 4.07 (quintet, *J* = 2.8 Hz, 1H), 3.93 (d, *J* = 13.4 Hz, 1H), 3.84 (d, *J* = 13.4, 1H), 3.76 (s, 6H), 3.15–3.09 (m, 4H), 2.85 (dd, *J* = 10.2, 2.4 Hz, 1H), 2.60 (dd, *J* = 10.2, 5.6 Hz, 1H), 2.41 (d quintet, *J* = 7.3, 3.3 Hz, 1H), 2.20 (t, *J* = 8.6 Hz, 1H) ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.6, 158.4, 151.2, 149.2, 145.0, 142.2, 136.2, 136.2, 133.2, 132.8, 130.4, 130.1, 129.0, 128.2, 127.8, 126.7, 115.8, 113.1, 86.0, 74.8, 64.6, 61.6, 56.2, 55.2, 48.8, 48.2. HRMS – found, 670.3026 calc for $C_{40}H_{40}N_5O_5$ $[M+H]^+$, 670.3029.

5.10. Compound 14

Compound **13** (1.00 g, 1.49 mmol) and MTET (0.259 g, 2.99 mmol) were dissolved, with warming, in dry MeCN (40 mL), evaporated to dryness and held under oil pump vacuum. **5** (1.01 g, 2.99 mmol) in dry MeCN (20 mL), was concentrated to dryness, held under oil pump vacuum and redissolved in dry CH_2Cl_2 (5.0 mL). This solution was added dropwise over 30 min to a solution of the **13**-MTET mixture in dry CH_2Cl_2 (5.0 mL). After a further1 h stirring at rt the reaction was quenched with Et_3N (0.63 mL, 4.48 mmol) and then hexanes (15 mL) was added and the mixture was applied directly to a silica gel column (25 × 4 cm), which had been washed with $CHCl_3$ – hexanes 1:1 containing 2% Et_3N. The column was eluted with this solvent and then with $CHCl_3$ followed by 70% EtOAc in $CHCl_3$ (both containing 2% Et_3N) to give **14** (1.02 g, 1.12 mmol, 75%) as a foam and a mixture of stereoisomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.88 (s, 1H), 9.15 (bs, 1H), 8.51 (s, 1H), 8.00 (m, 2H), 7.61 (m, 1H), 7.50 (m, 3H), 7.40 (m, 2H), 7.31–7.13 (m, 12H), 6.76 (m, 4H), 4.60 (m, 2H), 4.21 (m, 0.5H), 4.13 (m, 0.5H), 3.98 (dd, *J* = 3.1, 13.7 Hz, 1H) 3.87 (dd, *J* = 2.4, 13.7 Hz, 1H), 3.74 (m, 6H), 3.57 (m, 2H), 3.28 (dd, *J* = 5.3, 8.9 Hz, 0.5H), 3.23 (dd, *J* = 4.8, 8.6 Hz, 0.5H), 3.03 (m, 2H), 2.86 (dd, *J* = 6.6, 9.8 Hz, 0.5H), 2.82 (m, 1H), 2.75 (dd, *J* = 4.5, 9.8 Hz, 0.5H), 2.56 (m, 2H) 1.08 (m, 12H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.5, 158.3, 151.2, 149.2, 145.2, 142.1, 139.7, 136.4, 133.2, 132.8, 130.1, 128.5, 128.3, 128.2, 127.7, 127.0, 126.6, 115.8, 113.5, 113.0, 85.8, 75.6, 75.1, 65.3, 65.1, 64.3, 61.0, 56.4, 55.2, 48.5, 47.5, 43.0, 24.5. ^{31}P NMR ($CDCl_3$, 202 MHz) δ 147.3, 147.1.

5.11. Compound 15

Alcohol **11** (0.53 g, 0.540 mmol) and MTET (0.14 g, 1.62 mmol) were dissolved, with warming, in dry MeCN (40 mL) and evaporated to dryness. **14** (1.23 g, 1.35 mmol) in dry MeCN (10 mL), was concentrated to dryness and redissolved in dry CH_2Cl_2 (5.0 mL). This solution was added dropwise to a solution of the **11**-tetrazole mixture in dry CH_2Cl_2 (10.0 mL) held at −15°C under argon. The solution was stirred and allowed to warm to 0°C over 45 min *t*-BuOOH (70 mass% in H_2O, 0.37 mL, 2.70 mmol) was added and the solution was allowed to warm to rt. After 30 min the solution was washed with $NaHCO_3$ (10%, aq) dried and evaporated. Partial purification by flash chromatography gave a foam (1.04 g). This material was dissolved in 80% aq AcOH (20 mL), stirred at rt for 16 h and then evaporated. The residue was dissolved in $CHCl_3$ and washed with $NaHCO_3$ (10%, aq), dried and evaporated. Chromatography of the residue (0–20% MeOH in $CHCl_3$) gave **15** (0.465 g, 0.31 mmol, 57%) as a foam and a mixture of four stereoisomers. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.19 (br s, 1H), 11.05–10.66 (m, 2H), 9.74–9.05 (m, 2H), 8.81–8.23 (m, 2H), 8.09–7.70 (m, 3H), 7.66–7.19 (m, 20H), 6.20–6.10 (m, 1H), 5.74–5.63 (m, 1H), 5.56 (m, 1H), 5.18–4.67 (m, 7H), 4.52–4.03 (m, 5H), 4.01–3.16 (m, 15H), 3.12–2.15 (m, 5H), 1.24–1.09 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.6, 166.5, 165.2, 158.6, 155.6, 152.8, 151.7, 151.0, 150.0, 149.4, 149.2, 148.1, 147.5, 142.3, 141.8, 139.6, 135.1, 133.2, 133.1, 132.8, 132.7, 130.3, 129.2, 129.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.81, 127.75, 127.5, 127.0, 127.0, 123.8, 122.8, 116.0, 115.8, 113.2, 87.4, 87.0, 86.9, 81.5, 81.0, 80.7, 79.1, 78.5, 75.5, 72.2, 70.3, 69.8, 67.5, 66.3, 65.2, 63.7, 62.8, 60.3, 59.4, 58.9, 58.7, 55.5, 55.2, 48.7, 48.3, 47.8, 40.4, 35.8, 29.5, 22.8, 22.2, 19.2, 18.8. HRMS Found 1500.4386, calc for $C_{68}H_{73}ClN_{15}O_{19}P_2$ $[M+H]^+$, 1500.4371.

5.12. Compound 16

15 (0.44 g, 0.29 mmol) and **9** (0.665 g, 0.73 mmol) were coupled according to Method 1 using MTET (2 equiv) as the activator. After partial purification by chromatography (0–10% MeOH in $CHCl_3$) the residue was dissolved in AcOH (80%, 10 mL), stirred at rt for 16 h then evaporated, dissolved in $CHCl_3$, washed with aq $NaHCO_3$ dried and evaporated. Chromatography (0–25% MeOH in $CHCl_3$) gave **16** (35 mg, 0.018 mmol, 6%). ^{1}H NMR (CDCl3, 500 MHz) δ 12.24 (m, 2H), 10.75 (m, 2H), 9.58 (m, 2H), 8.73 (m, 1H), 8.57–8.18 (m, 2H), 8.14–7.76 (m, 6H),, 7.68–7.13 (m, 21H), 6.23–5.53 (m, 3H), 5.32–4.83 (m, 8H), 4.75–3.50 (m, 17H), 3.43–3.13 (m, 9H), 3.05–2.13 (m, 8H), 1.30–1.08 (m, 16H). ^{13}C NMR (CDCl3, 126 MHz) δ 180.1, 179.9, 179.6, 166.8, 166.6, 165.6, 165.3, 161.8, 155.9, 155.6, 152.5, 151.3, 151.0, 149.8, 149.3, 149.1, 148.5, 148.4, 148.1, 142.4, 141.8, 141.7, 140.6, 140.3, 140.0, 139.0, 138.2, 135.4, 135.2, 135.1, 133.5, 133.2, 132.8, 132.6, 130.6, 128.9, 128.7, 128.2, 128.1, 128.0, 127.3, 123.7, 122.2, 121.9, 121.4, 116.1, 115.9, 87.3, 87.2, 87.0, 86.8, 86.6, 86.1, 84.9, 84.7, 83.2, 83.0, 82.4, 82.2, 81.7, 81.5, 81.3, 79.9, 79.7, 79.5, 79.3, 75.6, 75.4, 75.0, 70.1, 69.9, 69.7, 69.1, 68.7, 67.8, 67.5, 67.1, 66.7, 61.5, 61.3, 61.1, 59.6, 59.2, 59.1, 59.0, 58.9, 58.8, 58.7, 58.5, 55.2, 54.3, 47.8, 47.6, 47.4, 47.3, 46.5, 46.2, 45.6, 36.0, 35.8, 19.2, 19.1, 19.0, 18.8. HRMS Found 1943.6171, calc for $C_{88}H_{98}N_{20}O_{26}P_3$ $[M+H]^+$, 1943.6174.

5.13. Compound 4

A solution of **16 (**0.035 g, 0.018 mmol) in THF (3 mL), and NH_3-MeOH (7 N, ~1 mL) was strried with Pd/C (10%) under H_2. After 40 h the solution was filtered through glass fibre paper and the solids were washed with THF – H_2O, (1:1) and then H_2O and all were evaporated. This material was dissolved in water, filtered through a 3 μm filter and lyophilized. A solution of this material in NH_4OH (27%, 5 mL) was stirred and heated in a sealed vessel at 40 °C for 18 h and then evaporated to dryness. The residue was purified by preparative HPLC and lyophilized (×4) to give **4** (5.3 mg, 0.004 mmol, 39%) as a non – stoichiometric Et_3 N salt ^{1}H NMR (500 MHz, D_2O) δ 8.38 (s, 1H), 8.34 (s, 1H), 8.20 (s, 1H), 8.01 (s, 1H), 7.94 (m, 2H), 6.15 (d, *J* = 4.9 Hz, 1H), 5.89 (d, *J* = 6.4 Hz, 1H), 5.84 (d, *J* = 4.5 Hz, 1H), 5.01 (m, 1H), 4.93 (m, 2H), 4.69 (m, 1H), 4.58 (m, 3H), 4.48–4.34 (m, 5H), 4.34–4.02 (m, 7H), 3.97–3.82 (m, 2H), 3.75 (m, 1H), 3.64 (m, 1H), 3.54–3.48 (m, 10 H), 2.99 (s, 1H); ^{13}C NMR (126 MHz, D_2O) δ 158.6, 158.2, 154.5, 153.6, 153.5, 152.2, 151.8, 151.2, 150.4, 148.4, 145.1, 139.7, 137.9, 137.1, 134.4, 118.3, 116.4, 116.0, 112.5, 101.9, 85.8, 85.6, 84.9, 83.74, 83.68, 83.0, 82.2, 81.2, 76.2, 73.0, 72.0, 68.7, 64.8, 64.7, 64.6, 61.2, 59.4, 58.3, 58.1, 57.9, 53.8, 46.8, 46.0. HRMS – found 1323.3246, $(M-H)^-$ calc for $C_{45}H_{58}N_{20}O_{22}P_3$ $[M-H]^-$ 1323.3247.

5.14. Phosphoramidite 17

A solution of 3-hydroxypropyl benzoate (1.5 g, 8.3 mmol, dried by evaporation from MeCN) in dry CH_2Cl_2 (30 mL) was added to an ice cooled solution of **5** (4.2 g, 12 mmol, 1.5 equiv) in dry CH_2Cl_2 containing suspended tetrazole (0.58 g, 8.3 mmol, 1 equiv). The suspension was stirred in the ice bath for 10 min and then allowed to warm to rt. After 20 min Et_3N (5 mL) was added and the solution was concentrated to approximately 15 mL. The resulting slurry was filtered through a silica gel plug that had been washed with 2% Et_3N in hexanes. The plug was washed with hexanes containing 2% of each of Et_3N and EtOAc. The resulting crude was further purified by chromatography using hexanes containing 2% Et_3N as eluent to give **17** as a colourless oil. (2.35 g, 5.8 mmol, 67%) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 2H), 7.29–7.21 (m, 4H), 7.16 (m, 1H), 4.68 (dd, $J_{H,P}$ = 8.3, $J_{H,H}$ = 12.6 Hz, 1H), 4.59 (dd, $J_{H,P}$ = 8.3, $J_{H,H}$ = 12.6 Hz, 1H), 4.36 (t, *J* = 6.3 Hz, 2H), 3.77 (m, 1H), 3.72 (m, 1H) 3.58 (m, 2H), 2.00 (q, *J* = 6.3 Hz, 2H), 1.12 (s, 3H), 1.12 (s, 3H), 1.11 s, (3H), 1.10 (s, 3H)· ^{13}CNMR ($CDCl_3$, 126 MHz) δ 166.6 (q), 139.5 (q), 132.8 (CH), 130.4 (q) 129.6, 128.3, 127.2, 127.0 (CH), 65.3 (CH_2, d, $J_{C,P}$ = 18 Hz), 62.0 (CH_2), 60.1 (CH_2, d, $J_{C,P}$ = 18 Hz), 43.0 (CH_2, d, $J_{C,P}$ = 13 Hz), 30.6 (CH_2), 24.6 (CH_3). ^{31}P NMR ($CDCl_3$, 202 MHz) δ 147.2. HRMS Found 418.2140, calc for $C_{23}H_{33}NO_4P$ $[M+H]^+$, 418.2147.

5.15. Compound 18

Alcohol **8** (2.0 g, 2.99 mmol) and phosphoramidite **15** (2.0 g, 2.2 mmol, 1.6 equiv) were coupled according to Method 1 using DCI (1.1 g, 9.0 mmol, 3 equiv) as the activator to give a pale coloured foam of **18** as a 1:1 mixture of diastereomers (2.2 g, 2.2 mmol, 74%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.92 (s, 1H), 8.92 (s, 0.5H), 8.91 (s, 0.5H), 7.97 (d, *J* = 8.0 Hz, 2H), 7.74 (s, 0.5H), 7.73 (s, 0.5H), 7.55 (m, 1H), 7.42–7.14 (m, 16H), 6.74 (m, 4H), 5.85 (dt, *J* = 7.7, 5.1 Hz, 0.5H), 5.80 (m, 1H), 5.76 (dt, *J* = 8.1, 4.5 Hz, 0.5H), 5.11 (d, *J* = 8.2 Hz,

2 × 0.5H), 4.95 (dd, *J* = 8.7, 11.8 Hz, 0.5H), 4.85 (m, 1.5H), 4.43 (m, 2× 0.5H), 4.32 (m, 1.5H), 4.24 (m, 2× 0.5H), 4.19 (m, 0.5H), 4.04 (m, 2× 0.5H), 3.75 (s, 1.5H), 3.74 (s, 1.5H), 3.73 (s, 1.5H), 3.72 (s, 1.5H),3.51 (m, 0.5H), 3.50 (s, 1.5H), 3.47 (dd, *J* = 2.3, 11.1 Hz, 0.5H), 3.40 (s, 1.5H), 3.15 (dd, *J* = 3.1, 11.1 Hz, 0.5H), 3.09 (dd, *J* = 3.1, 11.1 Hz, 0.5H), 2.10 (m, 2H), 1.98 (m, 1H), 1.06 (m, 3H), 0.93 (m, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 178.7, 166.6, 166.4, 158.7, 155.5, 147.9, 147.7, 147.5, 138.9, 138.7, 135.7, 135.4, 133.2, 129.9, 129.5, 128.8, 128.7, 128.4, 128.0, 127.9, 127.8, 127.1, 122.2, 113.2, 86.5, 86.4, 86.0, 85.6, 81.3, 81.1, 74.6, 74.4, 69.8, 69.6, 65.1,64.8, 62.3, 62.2, 61.0, 60.8, 58.7, 58.6, 55.5, 36.2, 29.5, 18.6. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.2, −1.3. HRMS – found 1002.3688, calc for $C_{53}H_{57}N_5O_{13}P$ $[M+H]^+$, 1002.3691.

5.16. Compound 19

18 (2.2 g, 2.2 mmol) was hydrolysed according to Method 2 to give **19** as a colourless foam and a mixture of stereoisomers (1.5 g, 2.2 mmol, 100%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.15 (s, 1H), 9.41 (s, 0.5H), 9.32 (s, 0.5H), 7.99 (d, *J* = 8.0 Hz. 2H), 7.82 (s, 0.5H), 7.78 (s, 0.5H), 7.56 (m, 1H), 7.45–7.29 (m, 7H), 5.75 (m, 1H), 5.35 (m, 1H), 5.14 (m, 2H), 4.86 (bm, 1H) 4.54 (m, 0.5H), 4.51 (m, 0.5H), 4.44 (m, 2H), 4.37 (s, 0.5H), 4.27 (m, 2H), 4.20 (s, 0.5H), 3.94 (d, *J* = 12.3 Hz, 0.5H), 3.87 (d, *J* = 12.3 Hz, 0.5H), 3.74 (m, 0.5H), 3.62 (m, 0.5H), 3.35 (s, 1.5H), 3.28 (s, 1.5H), 2.73 (quin, *J* = 7.0 Hz, 1H), 2.15 (m, 2H), 1.26 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.3, 166.7, 166.5, 155.5, 148.0, 147.8, 139.0, 135.5, 135.4, 133.2, 130.0, 129.5, 128.8, 128.7, 128.5, 128.0, 127.9, 121.9, 121.8, 87.2, 86.9, 84.9, 84.8, 81.8, 81.5, 75.8, 75.4, 69.9, 69.8, 65.1, 65.0, 61.5, 61.0, 60.9, 58.7, 58.5, 36.2, 29.6, 18.9. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.5, −1.6. HRMS- found 722.2197, calc for $C_{32}H_{39}N_5O_{11}PNa$ $[M+Na]^+$, 722.2203.

5.17. Compound 20

19 (0.70 g, 1.0 mmol) and tetrazole (70 mg, 1.0 mmol, 1.0 equiv) were dried by evaporation from MeCN. After redissolution in CH_2Cl_2 (10 mL) the mixture was added to a solution of **5** (0.68 g, 2 mmol, 2 equiv) in CH_2Cl_2 (5 mL). After stirring for 40 min Et_3N (1 mL) was added and the mixture concentrated to a small volume. Chromatography eluting with 20–40% EtOAc and 2% Et_3N in $CHCl_3$ gave **20** (0.8 g, 0.85 mmol, 85%) as a mixture of stereoisomers. ^{31}P NMR ($CDCl_3$, 202 MHz) δ 149.2, 149.1, 147.9, 147.7, −1.4, −1.5, −1.6.

5.18. Compound 21

20 (0.8 g, 0.85 mmol) was coupled with **13** (0.38 g, 0.56 mmol, 0.67 equiv) according to Method 1 using tetrazole (0.18 g, 3 equiv) as the activator to give **21** as a colourless foam (0.38 g, 0.24 mmol, 42% based on **13**). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.24–12.06 (m, 1H), 10.92 (m,1H), 10.78 (m, 1H), 8.98 (m, 1H), 8.56 (m, 1H), 8.02 (m, 4H), 7.61 (m, 2H), 7.54 (m, 3H), 7.45–7.10 (m, 22H), 6.77 (m, 4H), 5.69–5.59 (m, 1H), 5.17–4.92 (m, 5H), 4.84 (m, 1H), 4.68 (m, 1H), 4.45–4.33 (m, 3H), 4.30–4.10 (m, 4H), 3.99 (m, 1H), 3.90 (m, 1H), 3.74 (m, 7H), 3.60 (m, 1H), 3.27–2.98 (m, 5H), 2.85 (m, 1H), 2.68 (m, 1H), 2.56 (m, 1H), 2.21 (m, 1H), 2.07 (m, 2H), 1.20–0.91 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.9, 166.4, 158.5, 155.7, 151.1, 149.3, 148.1, 147.9, 144.8, 142.1, 140.1, 136.0, 133.2, 132.6, 130.1, 129.6, 129.1, 128.7, 128.4, 128.1, 127.8, 126.9, 123.0, 116.0, 113.1, 87.3, 86.2, 82.1,

81.4, 78.3, 75.2, 69.7, 66.6, 65.1, 63.3, 60.9, 59.3, 58.6, 55.9, 55.2, 48.0, 47.2, 35.7, 29.6, 19.2, 18.8. 31P NMR ($CDCl_3$, 202 MHz) δ −1.2, −1.5, −3.5. HRMS – found 1521.5349, calc for $C_{79}H_{83}N_{10}O_{18}P_2$ $[M+H]^+$, 1521.5362.

5.19. Compound 22

21 (0.38 g, 0.24 mmol) was hydrolysed according to Method 2 to give **22** (0.21 g, 0.18 mmol, 75%) as a colourless foam. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.24 (bs, 1H), 10.92 (bm, 1H), 10.73 (bm, 1H), 9.00 (bs, 1H), 8.56 (m, 1H), 8.02 (m, 4H), 7.61 (m, 2H), 7.54 (m, 3H), 7.45–7.10 (m, 13H), 5.60 (m, 1H), 5.33–4.94 (m, 6H), 4.94–4.67 (m, 2H), 4.49–4.04 (m, 6H), 4.01–3.65 (m, 2H), 3.55 (m, 1H), 3.46 (m, 1H) 3.31–3.16 (m, 3H), 3.09–2.86 (m, 1H), 2.73 (m, 1H), 2.69–2.48 (m, 2H), 2.33 (m, 2H), 2.11 (m, 2H), 1.24–1.08 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 180.0, 166.5, 155.7, 151.0, 149.3, 148.1, 142,2, 139.7, 135.4, 135.2, 133.2, 132.7, 130.2, 129.5, 129.1, 128.9, 128.8, 128.7, 128.5, 128.1, 127.9, 127.8, 122.9, 116.0, 87.6, 87.5, 81.8, 80.9, 79.2, 74.8, 69.8, 66.3, 65.0, 63.3, 60.9, 60.4, 59.7, 58.6, 55.2, 48.2, 47.8, 35.8, 29.5, 19.2, 18.8. 31P NMR ($CDCl_3$, 202 MHz) δ −1.2, −1.5, −2.7 HRMS – found 1219.4042 calc for $C_{58}H_{65}N_{10}O_{16}P_2$ $[M+H]^+$, 1219.4055. HPLC (Poroshell, 60–100% MeOH over 6 min) R_t 3.92, 3.97, 4.06, >90%.

5.20. Compound 23

22 (170 mg, 0.16 mmol) was deprotected according to Method 3. Chromatography of the crude on silica gel using 30–50% NH_4OH (7 M) in dioxane as the eluant followed by lyophilization gave the ammonium salt of **23** (42 mg, 52%) as a white solid. ^{1}H NMR (D_2O) δ 7.96 (s, 1H), 7.81 (s, 1H), 7.52 (s, 1H), 5.74 (d, *J* = 6.1 Hz, 1H), 4.84 (m, 1H), 4.64–4.61 (m, 1H), 4.40–4.36 (m, 1H), 4.38 – 4.34 (m, 1H), 4.31 (br quintet, *J* = 2.6 Hz, 1H), 4.25 (d, *J* = 13.9 Hz, 1H), 4.03 (dt, *J* = 12.0, 3.5 Hz, 1H), 3.97 (q, *J* = 6.4 Hz, 2H), 3.95–3.91 (m, 1H), 3.74 (dd, *J* = 12.0, 8.5 Hz, 1H), 3.67 (t, *J* = 6.4 Hz, 3H), 3.57 (brd, *J* = 5.5 Hz, 1H), 3.41 (s, 3H), 3.35 (brd, *J* = 12.4 Hz, 1H), 3.18–3.12 (m, 2H), 2.68 (br quintet, *J* = 6.4 Hz, 1H), 1.85 (quintet, *J* = 6.4 Hz, 2H). ^{13}C NMR (D_2O) δ 158.5, 153.7, 151.4, 150.3, 149.8, 143.8, 136.9, 131.5, 115.9, 113.4, 103.1, 85.1, 83.0, 81.4, 75.5, 72.3, 64.7, 63.2, 60.2, 58.3, 58.2, 58.0, 53.8, 47.8, 47.4, 32.5 (d, *J* = 6.6 Hz). 31P NMR (D_2O): δ = −0.11, −1.37. HRMS – found 759.2011, calc for $C_{26}H_{37}N_{10}O_{13}P_2$ $[M-H]^-$ 759.2017. HPLC (Kinetex, 0–30% MeCN over 15 min) R_t 8.18 min, 97%.

5.21. Compound 2

Alcohol **22** (0.090 g, 0.074 mmol) and amidite **17** (0.123 g, 0.3 mmol, 4 equiv) were coupled according to Method 1 using tetrazole (0.0063 g, 1.2 equiv) as the activator. The syrup (61 mg) obtained by chromatography was deprotected as described in Method 3. Chromatography of the crude on silica gel using 30–50% NH_4OH (7 M) in dioxane as the eluant followed by lyophilization gave the ammonium salt of **2** (19 mg, 44%) as a white solid that was further purified by preparative HPLC. ^{1}H NMR (D_2O) δ 8.30 (s, 1H), 7.99 (s, 1H), 7.80 (s, 1H), 5.91 (d, *J* = 6.4 Hz, 1H), 4.97 (m, 2H), 4.59 (m, 1H), 4.47 (m, 2H), 4.18 (dt, *J* =11.9, 4.0 Hz, 1H), 4.14–3.87 (m, 9H), 3.78 (t, *J* = 6.4 Hz, 2H), 3.74 (t, *J* = 6.4 Hz, 2H), 3.72 (dd, *J* = 4.2, 11.9 Hz, 0.5 H), 3.63 (dd, *J* = 6.5, 11.9 Hz, 0.5 H), 3.55 (bd, *J* = 13.2 Hz, 1H), 3.51 (s, 3H), 3.43 (d, *J* = 5.9 Hz, 1H), 3.41 (d, *J* = 5.4 Hz, 1H), 2.96 (brs, 1H), 1.96

(q, J = 6.3 Hz, 2H), 1.91 (q, J = 6.3 Hz, 2H). ^{13}C NMR (D_2O) δ 158.6, 153.7, 151.6, 150.6, 149.4, 143.2, 137.4, 132.2, 116.2, 113.6, 103.1, 85.3, 83.1, 81.1, 76.0, 72.4, 66.6, 65.0, 64.1, 63.1, 63.1, 58.6, 58.3, 58.2, 53.6, 48.3, 46.0, 32.5 (d, J = 7.0 Hz), 32.4 (d, J = 7.0 Hz). ^{31}P NMR (D_2O) δ 0.42, −0.07, −1.34. HRMS – found 897.2017, calc for $C_{29}H_{44}N_{10}O_{17}P_3$ [M – H]$^-$, 897.2099. HPLC (Poroshell, 0–350% MeOH over 5 min) R_t 2.01 min, >97%.

5.22. Compound 24

22 (0.21 g, 0.18 mmol) and phosphoramidite **9** (0.36 g, 0.2 mmol, 1.3 equiv) were coupled according to the Method 1 using tetrazole (0.020 g, 3 equiv) as the activator. The resulting colourless foam (0.18 g) was hydrolysed according to Method 3 to give **24** (0.085 g, 26%) as a pale foam. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.24 (bm, 2H), 10.87 (bm, 3H), 9.30 (bm, 1H), 8.56–7.05 (m, 29H), 5.87–5.60 (m, 2H), 5.45–3.50 (m, 25H), 3.50–3.12 (m, 8H), 2.90–2.35 (m, 6H), 2.09 (m, 2H), 1.33–1.00 (m, 12H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 180.1, 166.5, 155.7, 150.9, 149.4, 148.3, 142.5, 140.0, 138.9, 135.4, 135.1, 133.2, 132.6, 131.2, 129.5, 129.0, 128.7, 128.4, 128.1, 127.9, 122.3, 121.4, 115.9, 87.2, 86.5, 84.9, 81.7, 81.3, 79.2, 75.8, 74.6, 69.9, 66.8, 65.1, 61.3, 60.9, 58.6, 53.9, 47.5, 46.4, 35.8, 29.6, 19.2, 19.1. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.2, −2.1, −2.5, −3.5. HRMS – found 1738.5563, calc for $C_{80}H_{91}N_{15}O_{24}P_3$ [M+H]$^+$, 1738.5574. HPLC (Poroshell, 50–100% MeOH over 6 min) R_T 4.86–4.97, >88%.

5.23. Compound 3

24 (0.030 mg, 0.017 mmol) was hydrogenolysed and hydrolysed according to Method 3 and the resulting crude was purified on a plug of C-18 silica and then by preparative HPLC. The eluate was lyophilized to give **3** as a non – stoichiometric Et_3N salt (6.5 mg, 4.9 mmol, 29%). ^{1}H NMR (D_2O, 500 MHz) δ 8.20 (s, 1H), 8.02 (s, 1H), 7.98 (s, 1H), 7.77 (s, 1H), 5.92 (d, J = 6.2 Hz, 1H) 5.89 (d, J = 6.2 Hz, 1H), 5.00 (m, 1H), 4.95 (m, 1H), 4.91 (m, 1H), 4.58 (t, J = 5.8 Hz, 1H), 4.53 (m, 2H), 4.47 (m, 2H), 4.39 (m, 1H), 4.21–4.03 (m, 6H), 3.93 (m, 1H), 3.75 (t, J = 6.4 Hz, 2H), 3.71 (dd, J = 4.4, 11.8 Hz, 1H), 3.62 (m, 2H), 3.55–3.44 (m, 8H), 3.26 (q, J = 7.4 Hz, 10H, Et_3N), 2.99 (bs, 1H), 1.93 (m, 2H), 1.34 (t, J = 7.4 Hz, 15H, Et_3N). ^{13}C NMR (D_2O, 126 MHz) δ 158.7, 158.5, 153.7, 153.6, 151.6, 151.3, 150.4, 148.3, 137.8, 137.3, 132.7, 116.5, 116.1, 113.3, 103.1, 85.8, 85.3, 84.8, 83.1, 81.2, 76.0, 72.9, 72.4, 65.1, 64.6, 63.1, 62.6, 59.0, 58.3, 58.2, 58.1, 53.8, 48.5, 46.7, 46.0, 32.5, 8.3. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −0.1, −0.8, −1.3. HRMS-found 1118.2651 calc for $C_{37}H_{51}N_{15}O_{20}P_3$ [M−H]$^-$, 1118.2648. HPLC (Poroshell,-50% MeOH over 5 min) R_T 2.72, >97%.

5.24. Compound 25

24 (0.055 g, 0.032 mmol) and phosphoramidite **17** (0.053 g, 0.013 mmol, 4 equiv) were coupled according to Method 1 using tetrazole (0.011 g, 0.16 mmol, 5 equiv) as the activator. The resulting pale coloured foam (0.28 g) was hydrogenolysed and hydrolysed according Method 3 and the resulting crude was purified on a plug of C-18 silica and then by preparative HPLC. The eluate was lyophilized to give **25** (4.9 mg, 3.1 μmol, 6.4%) as a non – stoichiometric Et_3N salt contaminated with 5 mass% benzamide. ^{1}H NMR (D_2O, 500 MHz) δ 8.31 (s, 1H), 8.12 (s, 1H), 8.02 (s, 1H), 7.89 (s, 1H), 5.93 (d, J = 6.4 Hz, 1H) 5.91

(d, J = 6.6 Hz, 1H), 5.01 (m, 1H), 4.98 (m, 1H), 4.92 (m, 1H), 4.68 (m, 1H), 4.57 (m, 3H), 4.51 (m, 2H), 4.25–4.10 (m, 6H), 4.07 (q, J = 6.5 Hz, 2H), 3.92 (m, 3H), 3.77 (t, J = 6.4 Hz, 2H), 3.68 (bd, J = 13 Hz, 1H), 3.64 (t, J = 6.4 Hz, 2H), 3.57 (m, 1H), 3.50 (m, 7H), 3.27 (q, J = 7.4 Hz, 12H, Et_3N), 3.01 (bs, 1H), 1.94 (q, J = 6.4 Hz, 2H), 1.81 (q, J = 6.4 Hz, 2H), 1.35 (t, J = 7.4 Hz, 18H, Et_3N) ^{13}C NMR (D_2O, 126 MHz) δ 158.8, 158.6, 153.8, 151.9, 151.6, 150.5, 148.6, 138.1, 137.5, 132.7, 116.2, 113.0, 85.3, 84.9, 83.2, 81.1, 76.1, 73.2, 72.3, 65.1, 64.6, 63.1, 62.9, 59.1, 58.3, 58.2, 58.1, 53.9, 48.5, 46.8, 46.0, 32.5, 32.48.3. ^{31}P NMR ($CDCl_3$, 202 MHz) δ 0.3, 0.1, −0.9. HRMS- found 1256.273, calc for $C_{40}H_{58}N_{15}O_{24}P_4$ [M – H]$^-$,1256.2730, found 1256.2738. HPLC (Poroshell, 5–50% MeOH over 5 min) R_t 2.08, 96%.

5.25. Compound 26

BzCl (51 μL, 0.44 mmol) was added to a solution of **8** (0.24 g, 0.36 mmol) in pyridine (5 mL) and the resulting mixture stirred for 1 h under an atmosphere of argon. The reaction mixture was concentrated and dissolved in EtOAc (25 mL), washed with H_2O (10 mL) and NaHCO3 (10%, aq, 10 mL), dried and concentrated. The residue was purified by chromatography (50–100% EtOAc in hexanes) to afford a foam (0.28 g). which was dissolved in AcOH (80%, 5 mL) and stirred at rt for 15 min. Solvents were evaporated and the residue purified by chromatography on silica gel (EtOAc, then 10% MeOH in $CHCl_3$) to afford **26** (0.12 g, 63%) as a foam. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.09–8.06 (m, 2H), 7.98 (s, 1H), 7.60 (tt, J = 7.5, 1.2 Hz, 1H), 7.47 (t, J = 7.8 Hz, 2H), 5.90 (d, J = 6.9 Hz, 1H), 5.75 (dd, J = 5.2, 2.2 Hz, 1H), 4.70 (dd, J = 6.9, 5.2 Hz, 1H), 4.41 (q, J = 2.2 Hz, 1H), 4.03 (dd, J = 12.7, 2.6 Hz, 1H), 3.90 (brd, J =12.4 Hz, 1H), 3.29 (s, 3H), 2.78 (septet, J = 6.9 Hz, 1H), 1.28 (d, J = 4.7 Hz, 3H), 1.27 (d, J = 4.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.1, 165.7, 155.3, 147.9, 147.5, 139.1, 133.6, 129.8, 129.3, 128.6, 122.3, 83.4, 85.0, 81.5, 72.2, 62.3, 59.1, 36.3, 19.0, 18.9.

5.26. Compound 27

N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide-HCl (1.2 g, 6.0 mmol, 2.0 equiv.) and 4-dimethylaminopyridine (0.018 g, 0.15 mmol, 0.05 equiv) were added to a vigorously stirred solution of **8** (2.0 g, 3.0 mmol) and levulinic acid (0.71 g, 6.0 mmol, 2 equiv) in dry THF (50 mL). After 14 h the mixture was diluted with EtOAc and extracted with water, $KHSO_4$ (10%, aqueous), Na_2CO_3 (10%, aqueous) and brine. The organic phase was dried and concentrated to give crude levulinyl ester. This material was hydrolysed according to Method 2 to give **27** (1.2 g, 2.6 mmol, 90%) as a pale coloured foam. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.78 (s, 1H), 7.82 (s, 1H), 7.27 (s, 1H), 5.77 (d, J = 7.0 Hz, 1H), 5.55 (m, 1H), 5.27 (bs, 1H), 4.53 (dd, J = 5.3, 7.3 Hz, 1H), 4.28 (m, 1H), 3.95 (dd, J = 2.2, 12.6 Hz, 1H) 3.80 (d, J = 12.6 Hz, 1H), 3.28 (s, 3H), 287–2.67 (m, 5H), 2.20 (s, 3H), 1.28 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 206.4, 180.0, 172.1, 155.2, 147.8, 147.3, 139.0, 122.4, 88.2, 84.9, 81.4, 72.0, 62.4, 59.1, 37.9, 36.4, 29.8, 27.9, 18.9. HRMS – found 466.1936 calc for $C_{20}H_{28}N_5O_8$ [M+H]$^+$, 466.1938.

5.27. Compound 28

Alcohol **26** (0.078 g, 0.17 mmol) and phosphoramidite **14** (0.21 g, 0.23 mmol) were coupled according to Method 1 using tetrazole (0.023 g, 2 equiv) as the activator. The crude was purified by silica gel chromatography (4% Et_3N in EtOAc then 5% MeOH and 1% Et_3N in $CHCl_3$) and the residue stirred in AcOH (80%) for 4 h. Concentration and silica chromatography (2% Et_3N in MeCN – $CHCl_3$, 1:1 then 5% MeOH and 1% Et_3N in $CHCl_3$) gave **28** (0.16 g, 95%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.49 (s, 1H), 8.06–8.00 (m, 4H), 7.73 (s, 1H), 7.61 (dt, *J* = 7.4, 1.1 Hz, 2H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.42 (brt, *J* = 7.7 Hz, 2H), 7.39–7.32 (m, 6H), 5.75 (d, *J* = 7.6 Hz, 1H), 5.61 (dd, *J* = 5.3, 1.6 Hz, 1H), 5.10 (d, *J* = 9.0 Hz, 2H), 4.97 (dd, *J* = 7.5, 5.3 Hz, 1H), 4.77 (br septet, *J* = 3.0 Hz, 1H), 4.39–4.30 (m, 2H), 4.28–4.25 (m, 1H), 3.87 (d, *J* = 13.3 Hz, 1H), 3.69 (d, *J* = 13.3 Hz, 1H), 3.57 (dd, *J* = 10.7, 4.9 Hz, 1H), 3.52 (dd, *J* = 10.7, 5.7 Hz, 1H), 3.21 (s, 3H), 2.94 (dd, *J* = 8.8, 7.4 Hz, 1H), 2.77 (quintet, *J* = 6.8 Hz, 1H), 2.70 (dd, *J* = 10.9, 3.2 Hz, 1H), 2.63 (dd, *J* = 10.9, 5.6 Hz, 1H), 2.40–2.37 (m, 1H) 2.36–2.32 (m, 1H), 1.23 (d, *J* = 6.9 Hz, 3H), 1.19 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 180.1, 166.5, 165.7, 155.7, 1511, 149.3, 148.2, 148.1, 142.2, 139.8, 133.6, 133.2, 132.7, 130.1, 129.8, 129.1, 129.0, 128.9, 128.7, 128.6, 128.1, 127.8, 127.7, 123.0, 116.0, 112.4, 88.4, 81.6, 81.5, 81.0, 81.0, 79.1, 71.1, 69.9, 69.8, 66.8, 66.7, 63.4, 59.7, 59.0, 55.4, 48.3, 48.2, 47.9, 46.1, 35.8, 19.2, 18.8. HRMS – found 991.3508 calc for $C_{48}H_{52}N_{10}O_{12}P$ $[M+H]^+$, 991.3504.

5.28. Compound 29

A solution of **27** (0.16 g, 0.35 mmol) and phosphoramidite **14** (0.53 g, 0.58 mmol, 1.5 equiv) were coupled according to Method 1 using tetrazole (0.12 g, 1.7 mmol, 3 equiv) as the activator to give a pale coloured foam (0.38 g) which was hydrolysed according to Method 2 to give **29** (0.19 g, 0.19 mmol, 53%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.22 (bs, 1H), 11.00 (bs, 1H), 10.67 (bm, 1H), 9.03 (bs, 1H), 8.53 (s, 1H), 8.05 (m, 2H), 7.60 (m, 5H), 7.38 (m, 5H), 5.63 (dd, *J* = 8.2 Hz, 1H), 5.35 (m, *J* = 5.2 Hz, 1H), 5.17–4.77 (m, 4H), 4.39–3.82 (m, 5H), 3.53 (m, 2H), 3.19 (m, 4H), 2.93–2.55 (m, 7H), 2.40 (m, 1H), 2.19 (m, 4H), 1.29–1.10 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 206.4, 180.2, 172.2, 166.6, 156.0, 151.1, 149.7, 148.1, 142.6, 139.7, 133.4, 132.8, 129.1, 128.8, 128.2, 127.8, 127.7, 122.9, 115.9, 113.2, 88.1, 81.5, 78.8, 71.0, 70.1, 66.9, 59.0, 47.8, 37.8, 35.8, 29.8, 27.8, 19.2, 18.8. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −3.0. HRMS – found 985.3609 calc for $C_{46}H_{54}N_{10}O_{13}P$ $[M+H]^+$, 985.3609.

5.29. Compound 30

28 (0.16 g, 0.16 mmol) was deprotected according to Method 3. The crude product was washed with MeOH (20 mL) to give **30** (10 mg, 31%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.96 (s, 1H), 7.77 (s, 1H), 7.44 (s, 1H), 5.75 (d, *J* = 5.6 Hz, 1H), 4.55–4.51 (m, 1H), 4.71 (t, *J* = 4.5 Hz, 1H), 4.20 (t, *J* = 5.4 Hz, 1H), 4.15 (quintet, *J* = 3.0 Hz, 1H), 4.08 (d, *J* = 13.8 Hz, 1H), 3.99 (dt, *J* = 11.8, 3.5 Hz, 1H), 3.91 (dt, *J* = 11.8, 3.0 Hz, 1H), 3.85 (d, *J* = 13.8 Hz, 1H), 3.64 (dd, *J* = 11.4, 6.0 Hz, 1H), 3.57 (dd, *J* = 11.4, 6.5 Hz, 1H), 3.40 (s, 3H), 3.06 (d, *J* = 11.9 Hz, 1H), 2.73 (dd, *J* = 11.9, 5.2 Hz, 1H), 2.66 (t, *J* = 9.2 Hz, 1H), 2.59–2.53 (m, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.9, 154.0, 151.3, 150.3, 149.9, 144.7, 136.8, 130.3, 116.0, 113.4, 107.1, 85.2, 83.7 (d, *J* = 9.1 Hz), 82.8, 76.6 (d, *J* = 4.6 Hz), 68.8, 64.5

(d, *J* = 4.6 Hz), 61.1, 58.3, 58.2, 54.7, 48.0 (d, *J* = 5.3 Hz), 47.7. HRMS – found 623.2087 calc for $C_{23}H_{31}N_{10}O_9P$ $[M+H]^+$, 623.2091. HPLC (Kinetex, 0–50% MeCN, Rt 8.27 min, 84%).

5.30. Compound 31

Alcohol **28** (0.13 g, 0.13 mmol) and phosphoramidite **9** (0.26 g, 0.29 mmol, 2.2 equiv) were coupled according to Method A using tetrazole (0.023 g, 2.5 equiv) as the activator. Chromatography gave a white solid (0.11 g, HRMS – found 1812.6326, calc for $C_{91}H_{95}N_{15}O_{22}P_2$ $[M+H]^+$, 1812.6330) which was dissolved in AcOH (5 mL, 80%) and stirred at rt for 3 h. The solution was concentrated to dryness and the residue taken up in $CHCl_3$, washed with $NaHCO_3$ (10%, aq), dried and evaporated. Chromatography (0–20% MeOH in EtOAc) gave **31** as a white solid (58 mg, 0.0384 mmol, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.27 (s, 1H), 11.13–10.56 (m, 3H), 9.26 (s, 1H), 8.67–8.38 (m, 1H), 8.16–7.67 (m, 5H), 7.69–7.03 (m, 18H), 5.98–5.64 (m, 2H), 5.64–5.47 (m, 1H), 5.37–3.56 (m, 18H), 3.38–3.10 (m, 7H), 3.06–2.60 (m, 5H), 2.56–2.34 (m, 2H), 2.32–2.15 (m, 1H), 1.33–1.08 (m, 12H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 180.2, 180.1, 166.6, 165.6, 155.8, 155.7, 155.6, 151.1, 151.0, 149.2, 148.4, 148.33, 148.28, 148.1, 142.4, 142.3, 142.2, 140.24, 140.17, 139.2, 138.7, 138.4, 135.5, 135.4, 135.1, 133.7, 133.2, 132.64, 132.60, 130.6, 130.4, 130.3, 129.8, 129.7, 129.0, 128.9, 128.8, 128.7, 128.6, 128.2, 128.10, 128.06, 128.06, 128.03, 127.8, 127.4, 127.3, 122.6, 122.5, 121.8, 121.6, 121.5, 116.1, 116.0, 115.9, 112.3, 112.1, 88.4, 88.3, 86.6, 86.3, 85.2, 85.0, 81.5, 81.4, 80.1, 79.8, 79.6, 79.5, 79.3, 75.8, 71.4, 71.3, 69.9, 69.81, 69.76, 67.0, 61.5, 61.4, 60.4, 59.7, 59.3, 59.2, 59.0, 58.5, 54.5, 54.4, 54.3, 54.1, 47.7, 47.6, 46.6, 36.0, 35.9, 19.3, 19.11, 19.05, 18.9, 18.8. HRMS – found 1510.5031, calc for $C_{70}H_{78}N_{15}O_{20}P_2$ $[M+H]^+$, 1510.5023.

5.31. Compound 32

31 (0.055 g, 0.036 mmol) was deprotected according to Method 3. Silica chromatography (dioxane – NH_4OH, 1:1) gave clean **32** (8.0 mg, 8.1 μmol, 23%) as well as some slightly impure material (20 mg). ^{1}H NMR (D_2O, 500 MHz) δ 8.12 (s, 1H), 7.99 (s, 1H), 7.94 (s, 1H), 7.69 (s, 1H), 5.89–5.85 (m, 2H), 4.97 (s, 1H), 4.88 (s, 1H), 4.59–4.23 (m, 7H), 4.18–4.03 (m, 4H), 3.97–3.79 (m, 3H), 3.69–3.34 (m, 9H), 2.97 (s, 1H); ^{13}C NMR (D_2O, 126 MHz) δ 158.6, 158.4, 153.6, 151.2, 150.4, 149.8, 137.7, 137.0, 131.9, 116.5, 116.0, 113.5, 103.3, 85.7, 85.5, 84.9, 83.5, 83.4, 82.7, 81.3, 75.7, 73.0, 68.5, 64.8, 64.4, 61.2, 58.8, 58.2, 58.1, 53.7, 48.5, 45.9. HRMS – found 980.2565, calc for $C_{34}H_{44}N_{15}O_{16}P_2$ $[M-H]^-$, 980.2566, HPLC (Kinetex, 3–30% MeOH) R_t 7.42 min, 96%.

5.32. Compound 33

29 (0.09 g, 0.091 mmol) and **17** (0.095 g, 0.22 mmol, 2.5 equiv) were coupled according to Method 1 using tetrazole (0.026 g, 0.37 mmol, 4 equiv) as the activator to give a pale coloured foam (0.060 g) which was hydrolysed according to Method 3. The crude was purified on a plug of C-18 silica and then by preparative HPLC. The eluate was lyophilized to give **33** (8.0 mg, 10 mmol, 11%) as a pale foam. ^{1}H NMR (D_2O, 500 MHz) δ 8.10 (s, 1H), 7.86 (s, 1H), 7.63 (s, 1H), 5.80 (d, *J* = 5.7 Hz, 1H), 4.79 (s, 1H), 4.48 (t, *J* = 4.6 Hz, 1H), 4.44 (d, *J* = 13.0 Hz, 1H), 4.35 (d, *J* = 13.0 Hz, 1H), 4.28 (t, *J* = 5.3 Hz, 1H), 4.18 (m,

1H), 4.03 (m, 2H), 3.92 (m, 1H), 3.83 (m, 4H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.46 (d, *J* = 13.1 Hz, 1H), 3.41 (s, 3H), 3.36 (m, 1H), 3.29 (dd, *J* = 12.5, 6.2 Hz, 1H), 3.17 (q, *J* = 7.4 Hz, Et_3N), 2.84 (m, 1H), 1.79 (quin, *J* = 6.4 Hz, 2H), 1.25 (t, *J* = 7.4 Hz, Et_3N). ^{13}C NMR (D_2O, 126 MHz) δ 158.6, 153.7, 150.5, 149.8, 143.6, 137.1, 132.1, 116.1, 113.6, 103.1, 85.5, 83.6, 82.5, 75.8, 68.8, 63.9, 63.0, 58.5, 58.2, 53.5, 48.3, 46.7, 45.8, 32.4, 8.3. ^{31}P NMR (D_2O, 202 MHz) δ 0.5, −1.1. HRMS - found 759.2020 calc for $C_{26}H_{37}N_{10}O_{13}P_2$ $[M - H]^-$, 759.2017. HPLC (5–50% MeOH over 6 min) R_t 1.84 min, >99%.

5.33. Compound 34

27 (0.77 g, 1.65 mmol) and phosphoramidite **17** (1.0 g, 2.48 mmol, 1.5 equiv) were coupled according to Method 1 using DCI (0.59 g, 5 mmol, 3 equiv) as the activator to give **34** (0.65 g, 0.82 mmol, 50%) as a colourless foam and a mixture of stereoisomers. ^1H NMR ($CDCl_3$, 500 MHz) δ 12.20 (bs, 1H), 11.01 (bs, 0.5H), 10.90 (bs, 0.5H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.71 (s, 0.5H), 7.67 (s, 0.5H), 7.56 (m, 1H), 7.41 (m, 4H), 7.25 (m, 2H), 7.16 (m, 1H), 5.74 (d, *J* = 7.7 Hz, 0.5H), 5.68 (d, *J* = 7.7 Hz, 0.5H), 5.51 (d, *J* = 5.4 Hz, 0.5H), 5.39 (d, *J* = 5.4 Hz, 0.5H), 5.14 (d, *J* = 9.3 Hz, 1H), 5.01 (m, 1H), 4.94 (dd, *J* = 5.4, 7.7 Hz, 0.5H), 4.84 (dd, *J* = 5.4, 7.3 Hz, 0.5H), 4.46 (t, *J* = 6.0 Hz, 1H), 4.41–4.28 (m, 4H), 4.13 (m, 1H), 3.25 (s, 1.5H), 3.21 (s, 1.5H), 2.89–2.62 (m, 5H), 2.20 (m, 5H), 2.04 (m, 1H), 1.19 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 206.1, 179.8, 171.9, 171.8, 166.0, 155.5, 155.4, 147.9, 139.4, 135.0, 134.7, 132.9, 129.7, 129.3, 129.2, 128.7, 128.6, 128.4, 128.2, 127.9, 127.0, 122.7, 88.0, 81.3, 78.7, 70.7, 68.8, 69.3, 66.8, 66.6, 64.8, 64.5, 60.5, 60.4, 58.8, 37.6, 35.4, 29.5, 29.4, 29.2, 27.86, 18.9, 18.8, 18.6. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −2.5, −2.6. HRMS – found 798.2749 calc for $C_{37}H_{45}N_5O_{13}P$ $[M+H]^+$, 798.2752.

5.34. Compound 35

Hydrazine hydrate (0.1 mL, 50%) was added to a solution of **34** (0.57 g, 0.72 mmol) in MeOH (5 mL). After 5 h the reaction was quenched with excess acetone and concentrated. Chromatography of the residue, eluting with a gradient of methanol (0–10%) in $CHCl_3$ – EtOAc, 2:1, gave **35** (0.35 g, 0.50 mmol, 70%). as a pale coloured foam and a mixture of stereoisomers. ^1H NMR ($CDCl_3$, 500 MHz) δ 12.30 (bs, 0.5H), 12.24 (bs, 0.5H), 11.06 (bs, 0.5H), 10.94 (bs, 0.5H), 8.01 (m, 1H), 7.95 (m, 1H), 7.79 (s, 0.5H), 7.76 (s, 0.5H), 7.55 (m, 1H), 7.39 (m, 4H), 7.25 (m, 2H), 7.18 (m, 1H), 5.88 (d, *J* = 6.3 Hz, 0.5H), 5.82 (d, *J* = 6.1 Hz, 0.5H), 5.14 (m, 1H), 5.00 (m, 1H), 4.57 (m, 2H), 4.45 (t, *J* = 6.0 Hz, 1H), 4.33 (m, 4H), 4.13 (m, 1H), 3.97 (bs, 1H), 3.34 (s, 1.5H), 3.32 (s, 1.5H), 2.86 (m, 0.5H), 2.79 (m, 0.5H), 2.18 (m, 1H), 2.04 (m, 1H), 1.22 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 180.2, 180.1, 166.3, 166.2, 155.6, 148.1, 139.2, 135.2, 135.0, 133.0, 129.8, 129.7, 129.4, 128.8, 128.6, 128.5, 128.3, 128.0, 127.2, 122.4, 88.1, 88.0, 83.4, 83.4, 81.3, 69.8, 69.5, 69.4, 67.4, 67.2, 64.9, 64.6, 60.7, 60.5, 60.3, 58.5, 35.6, 29.5, 29.4, 19.0, 18.9, 18.8.^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.9. HRMS – found 700.2377 calc for $C_{32}H_{39}N_5O_{11}P$ $[M+H]^+$, 700.2384.

5.35. Compound 36

A solution of **35** (0.18 g, 0.26 mmol) and DCI (0.036 g. 0.31 mmol, 1.2 equiv) in dry CH_2Cl_2 (5 mL) was added dropwise to a solution of **5** (0.13 g, 0.31 mmol, 1.2 equiv) in dry CH_2Cl_2 (2 mL). After 1 h the reaction was quenched with Et_3N (1 mL) and concentrated.

Column chromatography of the residue, (hexanes – $CHCl_3$, 1:1 then $CHCl_3$, both with 1% Et_3N) gave **36** (0.25 g, 0.26 mmol, 86%), ^{31}P NMR ($CDCl_3$, 202 MHz) δ 151.6, 149.6, −2.0, −1.7.

5.36. Compound 37

13 (0.10 g, 0.15 mmol) and phosphoramidite **17** (0.076 g, 0.18 mmol, 1.2 equiv) were coupled together according to Method 1 using tetrazole (0.021 g, 0.30 mmol, 2 equiv) as the activator. The resulting pale foam (0.11 g) was hydrolysed according to Method 2 to give **37** (0.33 g, 0.48 mmol, 66%) as a colourless foam and a mixture of diastereomers. ^1H NMR ($CDCl_3$, 500 MHz) δ 10.95 (bs, 1H), 9.33 (bs, 1H), 8.53 (s, 1H), 7.99 (m, 4H), 7.62 (m, 1H), 7.53 (m, 4H), 7.41 (m, 2H), 7.32 (m, 5H), 5.04 (m, 2H), 4.81 (m, 0.5H), 4.77 (m, 0.5H) 4.34 (m, 2H), 4.16 (m, 2H), 3.93 (dd, *J* = 13.6, 6.3 Hz, 1H), 3.86 (dd, *J* = 13.7, 8.1 Hz, 1H), 3.61 (m, 2H), 3.18 (bs, 1H), 3.01 (dd, *J* = 10.7, 6.5 Hz, 0.5H), 2.93 (m, 1.5H), 2.76 (dd, *J* = 10.7, 3.6 Hz, 0.5H), 2.72 (dd, *J* = 10.7, 3.6 Hz, 0.5H), 2.45 (m, 2H), 2.07 (m, 2H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.6, 166.2, 150.9, 149.1, 142.3, 135.6, 135.7, 133.0, 132.9,132.7130.1, 129.9, 129.4, 128.8, 128.4, 128.2, 127.8, 127.7, 115.8, 112.5, 80.3, 69.3, 64.3, 63.6, 60.8, 60.1, 55.4, 48.4, 47.7, 29.4. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.2, for −1.3. HRMS- found 700.2534 calc $C_{36}H_{39}N_5O_8P$ $[M+H]^+$, 700.2536.

5.37. Compound 38

36 (0.18 g, 0.19 mmol) was coupled with **37** (0.094 g, 0.14 mmol, 0.7 equiv) according to Method 1 using tetrazole (0.04 g, 0.58 mmol, 3 equiv) as the activator to give **38** (0.050 g, 0.033 mmol, 32%) as a pale coloured foam and a mixture of stereoisomers.^1H NMR ($CDCl_3$, 500 MHz) δ 12.17 (m, 1H), 10.90 (m, 2H), 9.12 (bs, 1H), 8.57 (s, 1H), 7.98 (m, 6H), 7.72 (m, 1H), 7.54 (m, 5H), 7.45–7.17 (m, 18H), 7.11 (m, 1H), 5.73 (m, 1H), 5.10 (m, 7H), 4.82 (m, 1H), 4.69 (m, 1H), 4.51–4.02 (m, 13H), 3.90 (m, 2H), 3.22 (m, 3H), 3.00–2.57 (m, 5H), 2.39 (m, 2H), 2.14 (m, 2H), 1.98 (m, 2H), 1.56 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.9, 166.4, 155.6, 151.2, 149.3, 148.1, 142.2, 139.5, 135.6, 135.2, 135.0, 133.3, 133.2, 133.1, 132.6, 130.3, 129.9, 129.8, 129.5, 129.1, 128.9, 128.8, 128.7, 128.6, 128.4, 128.1, 127.9, 127.7, 127.2, 122.7, 115.8, 112.4, 87.1, 82.2, 82.0, 79.4, 79.0, 78.7, 75.1, 69.9, 69.8, 69.5, 67.7, 66.8, 65.0, 64.6, 60.9, 60.7, 59.8, 58.7, 58.6, 54.6, 47.6, 46.6, 35.8, 29.5, 19.1, 18.9, 18.8. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.1, −1.3, −1.5, −1.6, −1.7, −1.8, −1.9, −2.0, −2.1.

5.38. Compound 39

38 (0.045 g, 0.029 mmol) was deprotected according to Method 3. Chromatography on C-18 silica (10% MeOH in aqueous Et_3NHOAc (0.05 M) gave **39** (0.015 g, 0.013 mmol, 43%) as a non – stoichiometric Et_3N salt contaminated with 10 mass% $BzNH_2$. ^1H NMR (D_2O, 500 MHz) δ 8.13 (s, 1H), 8.08 (s, 1H), 7.58 (s, 1H), 5.88 (d, *J* = 6.7 Hz, 1H), 4.93 (m, 1H), 4.50 (m, 3H), 4.09 (m, 3H), 3.94 (m. 7H), 3.69 (dt, *J* = 6.4, 3.0 Hz, 2H), 3.62 (t, *J* = 6.4 Hz, 2H), 3.35 (s, 3H), 3.18 (q, *J* = 7.3 Hz, Et_3N), 3.04 (m, 1H), 2.93 (dd, *J* = 6.0, 10.8 Hz, 1H), 2.62 (m, 1H), 2.51 (dd, *J* = 7.8, 9.7 Hz, 1H), 1.84 (quin, *J* = 6.4 Hz, 2H), 1.80 (quin, *J* = 6.4 Hz, 2H), 1.29 (t, *J* = 7.3 Hz, Et_3N). ^{13}C NMR (D_2O, 126 MHz) δ 163.3, 157.4, 151.9, 150.3, 149.8, 145.3, 136.2, 132.5, 130.0, 128.7, 127.4, 117.0, 113.3, 109.3, 84.5, 83.4, 81.3, 76.3,

73.1, 65.9, 65.2, 62.9, 62.7, 59.3, 58.2, 57.9, 54.0, 46.6, 32.4, 8.3. ^{31}P NMR ($CDCl_3$, 202 MHz) δ 0.4, −0.1, −0.6. HRMS – found 897.2098 calc for $C_{29}H_{44}N_{10}O_{17}P_3$ $[M+H]^+$, calculated 897.2099. HPLC (Poroshell, 0–30% MeCN over 12 min) R_t 4.25 min, >98%.

5.39. Compound 40

37 (0.26 g, 0.37 mmol) and phosphoramidite **9** (0.67 g, 0.74 mmol 2 equiv) were coupled according to Method 1 using tetrazole (0.078 g, 1.1 mmol, 3 equiv) as activator. The resulting pale foam (0.34 g) was hydrolysed according to Method 2 to give **40** (0.22 g, 0.18 mmol, 50%) as a colourless foam and a mixture of stereoisomers ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.16 (bs, 1H), 10.92 (bs, 1H), 9.86 (m, 1H), 8.92 (bs, 1H), 8.57 (m, 1H), 8.08–7.86 (m, 5H), 7.67–7.25 (m, 17H), 5.84 (m, 1H), 5.41–5.17 (m, 1H), 5.07 (m, 4H), 4.69 (m, 1H), 4.52 (m, 1H), 4.33 (m, 3H), 4.12 (m, 4H), 3.96–3.58 (m, 4H), 3.30 (m, 4H), 2.92 (m, 2H), 2.81 (m, 1H), 2.64 (m, 2H), 2.44 (m, 1H), 2.04 (m, 2H), 1.20 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 179.6, 166.3, 155.4, 151.1, 149.4, 148.2, 142.2, 138.5, 135.6, 133.3, 133.1, 132.6, 130.2, 129.5, 129.1, 128.6, 128.4, 128.1, 127.9, 127.6, 115.8, 86.0, 84.9, 84.1, 81.7, 78.7, 76.5, 75.4, 69.6, 67.4, 64.7, 61.5, 60.9, 59.7, 58.8, 54.4, 47.6, 46.3, 35.9, 29.6, 18.9. 31P NMR ($CDCl_3$, 202 MHz) δ −1.5, −1.6, −1.7, −2.1, −2.4. HRMS – found 1219.4041 calc for $C_{58}H_{65}N_{10}O_{16}P_2$ $[M+H]^+$, 1219.4055.

5.40. Compound 41

40 (0.22 g, 0.18 mmol) and phosphoramidite **9** (0.34 g, 0.37 mmol 2 equiv) were coupled according to Method 1 using tetrazole (0.039 g, 0.56 mmol, 3 equiv) as activator to give the protected triphosphate (0.23 g, 0.12 mmol, 66%). HRMS – found 2040.6893 calc for $C_{101}H_{109}N_{15}O_{26}P_3$ $[M+H]^+$, 2040.6881. Acidic hydrolysis (Method 2) and hydrogenolysis and basic hydrolysis (Method 3) gave a crude material that was purified first on a plug of C-18 silica and then by preparative HPLC. The eluate was lyophilized to give **41** (1.5 mg, 1.1 μmol, 0.6%) a non – stoichiometric Et_3N salt. ^{1}H NMR (D_2O, 500 MHz) δ 8.10 (s, 1H), 8.00 (s, 1H), 7.90 (s, 1H), 7.65 (s, 1H), 5.81 (m, 2H), 4.92 (m, 1H), 4.81 (m, 1H), 4.67 (m, 1H), 4.57 (m, 1H), 4.45 (m, 1H), 4.39 (m, 3H), 4.25 (m, 1H), 4.16 (m, 2H), 4.02 (m, 2H), 3.87 (m, 2H), 3.69 (m, 3H), 3.60 (m, 2H), 3.50 (m, 1H), 3.45 (m, 1H), 3.39 (s, 3H), 3.33 (s, 3H) 3.18 (q, J = 7.3 Hz, Et), 3.15 (m, 1H), 2.82 (m, 1H), 1.76 (quin, J = 6.4 Hz, 2H), 1.26 (t, J = 7.3 Hz, Et_3N). ^{13}C NMR (D_2O, 126 MHz, excluding quaternary carbons) δ 150.9, 138.2, 132.0, 86.4, 84.9, 83.7, 81.7, 76.4, 73.3, 65.5, 63.5, 61.5, 59.7, 58.5, 54.3, 48.5, 47.3, 32.9, 8.8. 31P NMR (D_2O, 202 MHz) δ −0.5, −0.8, −1.0. HRMS – found 1120.2810, calc for $C_{37}H_{53}N_{15}O_{20}P_3$ $[M+H]^+$, 1120.2804. HPLC (Poroshell, 50–100% MeOH over 6 min) R_t 2.76 min, 97%.

5.41. Compound 42

MsCl (0.40 mL, 1.9 equiv) and then Et_3N (0.80 mL, 2.1 equiv) were added to a stirred solution of 6-*N*-benzoyl-9-deaza-9-(2-hydroxyethyl)adenine (0.75 g, 2.7 mmol) [18] in CH_2Cl_2 (100 mL). After 4 h the solution was washed with H_2O and $NaHCO_3$ (10%, aq). The organic phase was dried and evaporated to a white solid (0.90 g) which was taken up in DMF (30 mL). (3*R*, 4R)-4-Hydroxymethylpyrrolidin-3-ol [17] (0.55 g, 4.7 mmol) and Na_2CO_3 (0.75 g) were added and the solution stirred at 90 °C. The solvent was evaporated

under reduced pressure and the residue suspended in $CHCl_3$ – MeOH, 9:1 and filtered. Concentration of the solvent and chromatography of the residue (MeOH with 1% NH_4OH (0–60%) in $CHCl_3$) gave **42** (0.78 g, 2.1 mmol, 76%). ^1H (NMR $CDCl_3$-CD_3OD, 2:1, 500 MHz) δ 8.56 (s, 1H), 8.08 (d, *J* = 7.1 Hz, 2H), 7.66 (m, 1H), 7.58 (m, 3H), 4.13 (dt, *J* = 3.7, 5.9 Hz, 1H), 3.66 (dd, *J* = 6.3, 10.8 Hz, 1H), 3.61 (dd, *J* = 6.5, 10.9 Hz, 1H) 3.15 (dd, *J* = 8.2, 10.0 Hz, 1H), 3.06 (t, *J* = 7.6 Hz, 2H), 2.92 (m, 3H), 2.81 (dd, *J* = 3.6, 10.3 Hz, 1H), 2.51 (dd, *J* = 6.5, 9.7 Hz, 1H), 2.27 (m, 1H). ^{13}C NMR $CDCl_3$-CD_3OD, 2:1, 126 MHz) δ 166.7, 149.9, 148.1, 142.6, 132.7, 132.3, 129.1, 128.5, 127.6, 115.9, 113.0, 72.8, 62.8, 61.6, 56.0, 55.5, 49.2, 22.1. HRMS – found 382.1878 calc for $C_{20}H_{24}N_5O_3$ $[M+H]^+$, 382.1879.

5.42. Compound 43

A solution of alcohol **42** (0.77 g, 2.0 mmol) in dry pyridine was concentrated to dryness, held under oil pump vacuum for 0.5 h and then redissolved in dry pyridine (5 mL) to which DMTrCl (1.0 g, 1.5 equiv) was added. The solution was stirred for 16 h and then partitioned between $CHCl_3$ and H_2O. The organic phase was dried, concentrated and the residue purified by chromatography (MeOH (0–10%) in $CHCl_3$) to give **43** (0.45 g, 33%). A quantity of ditritylated material was also recovered that was converted to the desired monotrityl product by stirring for 40 min in MeOH- HCl. ^1H NMR ($CDCl_3$, 500 MHz) δ 10.8 (bs, 1H), 9.03 (bs, 1H), 8.56 (s, 1H), 8.00 (d, *J* = 7.1 Hz, 2H), 7.64 (tt, *J* = 7.4, 1.8, 1H), 7.54 (m, 2H), 7.43 (m, 3H), 7.33–7.25 (m, 6H), 7.23 (m, 1H), 6.82 (m, 4H), 4.06 (m, 1H), 3.78 (s, 6H), 3.13 (m, 2H), 3.02 (t, *J* = 7.6 Hz, 2H), 2.82 (m, 3H), 2.57 (dd, *J* = 5.4, 9.8 Hz, 1H), 2.39 (m, 1H) 2.15 (dd, *J* =7.9, 9.6, 1H), 2.11 (bs, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.3, 158.5, 151.2, 148.9, 142.0, 136.3, 133.2, 132.8, 130.1, 129.1, 128.7, 128.2, 127.8, 127.7, 126.7, 115.9, 114.8, 113.1, 86.0, 74.8, 64.7, 61.8, 56.2, 56.0, 55.2, 48.7, 23.1. HRMS -found 684.3176 calc for $C_{41}H_{42}N_5O_5$ $[M+H]^+$, 684.3186.

5.43. Compound 44

43 (0.17 g, 0.24 mmol) and phosphoramidite **20** (0.33 g, 0.35 mmol, 1.5 equiv) were coupled according to the Method 1 using tetrazole (0.074 g, 3 equiv) as the activator. Chromatography gave of a glassy solid (0.28 g. HRMS – found 1535.5515 calc for $C_{80}H_{85}N_{10}O_{18}P_2$ $[M+H]^+$, 1535.5519) which was hydrolysed according to Method 2 to give **44** (0.12 g, 0.10 mmol, 40%) as a mixture of diastereomers. ^1H NMR ($CDCl_3$, 500 MHz) δ 12.2 (bs, 1H), 10.8 (bs, 2H), 9.21 (bs, 1H), 8.48 (s, 1H), 8.00 (m, 4H), 7.63 (m, 2H), 7.54 (m, 3H), 7.45–7.28 (m, 15H), 5.68 (m, 1H), 5.11 (m, 5H), 4.81 (m, 2H), 4.41 (m, 3H), 4.25 (m, 4H), 3.53 (m, 2H), 3.31–3.20 (m, 4H), 3.13–2.60 (m, 7H), 2.42 (m, 1H), 2.12 (m, 2H) 1.2 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 180.1, 166.4, 155.6, 150.7, 148.8, 148.1, 142.3, 139.7, 135.3, 133.2, 132.7, 129.9, 129.5, 129.0, 128.8, 128.5, 128.2, 128.1, 127.9, 127.8, 122.8, 115.9, 87.6, 81.8, 80.6, 79.2, 74.6, 70.0, 66.6, 65.1, 62.4, 60.9, 60.1, 58.6, 55.6, 55.0, 47.9, 35.8, 29.6, 22.5, 19.2, 18.9. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.2, calc −1.4, −2.8. HRMS – found 1233.4215 calc for $C_{59}H_{67}N_{10}O_{16}P_2$ $[M+H]^+$, 1233.4212.

5.44. Compound 45

44 (0.11 g, 0.089 mmol) and **17** (0.074 g, 0.52 mmol, 2.0 equiv) were coupled according to Method 1 using tetrazole (0.025 g, 4 equiv) as the activator. Chromatography gave a glassy

solid (0.040 g, (HRMS – found 1565.5018 calc for $C_{76}H_{84}N_{10}O_{21}P_3$ $[M + H)]^+$, 1565.5025). This material was deprotected according to Method 3 and purified on a column of C-18 silica (MeOH (15%) in Et_3NHOAc (50 mM, pH 6). Lyophilization gave **45** (0.011 g, 7.7 μM, 8.6%) as a non-stoichiometric Et_3NH^+ salt contaminated with 15 mol% $BzNH_2$. ^1H NMR (D_2O, 500 MHz) δ 8.13 (s, 1H), 8.03 (s, 1H), 7.40 (s, 1H), 5.88 (d, *J* = 5.8, 1H), 5.00 (dt, *J* = 4.6, 8.6, 4.96 (m, 1H), 4.53 (m, 2H), 4.28 (dm, *J* = 11.6, 1H), 4.21 (dt, *J* = 11.6, 3.7, 1H), 4.09 (q, *J* = 6.35, 3H), 4.03 (q, *J* = 5.8, 3H), 3.92 (dd, *J* = 9.0, 12.2, 3.77 (m, 3.52 (s, 3H), 3.44 (m, 3H), 3.25 (m, Et_3N + 1H), 2.96 (m, 3H), 1.95 (m, 4H), 1.33 (m, Et_3N). ^{13}C NMR (D_2O, 126 MHz) δ 158.1, 153.4, 151.2, 150.2, 148.4, 137.2, 128.8, 128.7, 127.4, 115.8, 113.1, 109.5, 85.2, 82.8, 81.4, 76.2, 72.3, 65.3, 64.2, 63.2, 63.1, 62.5, 59.5, 58.3, 58.2, 55.4, 54.0, 46.7, 45.7, 32.5, 20.7, 8.3. ^{31}P NMR (D_2O, 202 MHz) δ −0.5, −0.1, −1.1. HRMS – found 935.2196 calc for $C_{30}H_{47}N_{10}O_{17}P_3Na$ $[M+Na]^+$, 935.2197. HPLC (Poroshell, 0–50% MeOH over 5 min) R_t 2.49 min, > 95%.

5.45. Compound 47

9-Deazaadenine [20] (1.49 g, 1.1 equiv), and then formaldehyde (aq, 37 mass%, 1.56 mL, 2 equiv) were added to a solution of amine **46** [21] (2.38 g, 10.1 mmol) in EtOH (20 mL) and H_2O (10 mL). The resulting suspension was warmed to 60 °C and stirred for 2 h. Silica gel (10 g) was added, solvents evaporated and the resulting solid purified by chromatography (10% 7 N NH_3 in MeOH – $CHCl_3$) to afford the title compound **47** (2.00 g, 52%) as a white solid. ^1H NMR (CD_3OD, 500 MHz) δ 8.17 (s, 1H), 7.46 (s, 1H), 7.33–7.17 (m, 5H), 3.90 (dd, *J* = 11.8, 4.3 Hz, 2H), 3.80 (s, 2H), 3.60 (s, 2H), 3.53 (dd, *J* = 11.8, 7.3 Hz, 2H), 2.42 (d, *J* = 7.5 Hz, 2H), 2.02–1.95 (m, 1H), 1.32 (s, 3H), 1.20 (s, 3H). ^{13}C NMR (d_4-MeOH, 126 MHz) δ 152.0, 150.8, 147.2, 141.0, 130.0, 129.7, 129.2, 127.9, 115.2, 113.8, 99.2, 64.1, 60.2, 53.6, 48.6, 33.8, 25.4, 23.3.

5.46. Compound 48

A suspension of **47** (2.0 g, 5.2 mmol) and $Pd(OH)_2$ – C (0.5 g) in a mixture of MeOH (20 mL) and NH_4OH (2 mL) was stirred under a balloon of hydrogen for 48 h. Filtration through diatomaceous earth and evaporation of solvent gave a residue that was absorbed onto silica gel (10 g) and purified by chromatography (20% 7 N NH_3 in MeOH – $CHCl_3$) to afford **48** (1.39 g, 93%) as a white solid. ^1H NMR (CD_3OD, 500 MHz) δ 8.15 (s, 1H), 7.47 (s, 1H), 3.94 (dd, *J* = 1.8, 4.3 Hz, 2H), 3.91 (s, 2H), 3.66 (dd, *J* = 11.8, 7.3 Hz, 2H), 2.62 (d, *J* = 6.9 Hz, 2H), 1.94–1.87 (m, 1H), 1.35 (s, 6H). ^{13}C NMR (d_4-MeOH, 126 MHz) δ 152.1, 150.9, 146.6, 129.1, 115.4, 114.4, 99.3, 64.1, 49.3, 49.0, 44.0, 35.8, 25.6, 23.0. HRMS – found 292.1776 calc for $C_{14}H_{22}N_5O_2$ $[M+H]^+$, 292.1773.

5.47. Compound 49

Fmoc chloride (3.40 g, 13 mmol) was added to a rapidly stirred suspension of **48** (2.5 g, 8.6 mmol) in MeOH (250 mL) and $NaHCO_3$ (10% aq, 25 mL). After stirring overnight silica gel (250 g) was added, solvents evaporated and the resulting solid purified by chromatography (20% MeOH in $CHCl_3$) to afford a gum (2.8 g) which was committed to the next step without characterization. BzCl (1.9 mL, 16 mmol) was added dropwise to a solution of this gum in pyridine (50 mL) and the resulting mixture stirred for 48 h. Solvent was evaporated

and the residue partitioned between $CHCl_3$ and $NaHCO_3$ (10% aq). The organic layer was separated and washed with water, dried and concentrated onto silica (20 g) and purified by chromatography ($CHCl_3$ then EtOAc then 5% MeOH in $CHCl_3$) to afford a gum (2.24 g) which was committed to the next step without characterization. The gum was dissolved in THF (40 mL), AcOH (20 mL) and H_2O (20 mL) and stirred for 48 h at rt and then concentrated. The resulting syrup was dissolved in $CHCl_3$ (200 mL) and washed with $NaHCO_3$ (10% aq) and brine. The organic layer was dried and concentrated and the resulting residue purified by chromatography. (EtOAc then 10% MeOH in $CHCl_3$) to give a white foam (2.1 g) which was committed to the next step without characterization. DMTrCl (1.26 g, 3.6 mmol) was added to this foam dissolved in pyridine (25 mL) and the solution stirred for 24 h. The mixture was concentrated and the residue dissolved in $CHCl_3$, washed with water, HCl (10% aq) and $NaHCO_3$ (10% aq), The organic phase was dried and concentrated and the resulting residue purified by chromatography (50–100% EtOAc in hexanes) to afford **49** (2.61 g, 3.0 mmol, 35% over 4 steps) as a foam. ^1H NMR ($CDCl_3$, 500 MHz) δ 10.80 (m, 1H), 9.24 (s, 1H), 8.44 (m, 1H), 7.99 (m, 2H), 7.84–7.07 (m, 20H), 6.76 (m, 4H), 6.55 (s, 1H), 4.79–4.33 (m, 3H), 4.24 (m, 2H), 3.73 (s, 6H), 3.64–3.22 (m, 5H), 3.21–2.99 (m, 2H) 2.03 (s, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.6, 158.4, 157.4, 156.5, 150.8, 149.9, 149.4, 145.1, 144.0, 142.0, 141.5, 136.2, 136.1, 135.9, 133.3, 132.8, 131.3, 130.1, 129.1, 128.2, 127.7, 127.7, 127.2, 126.6, 124.9, 124.7, 123.7, 120.1, 119.9, 115.4, 113.0, 112.7, 86.1, 66.7, 63.6, 63.2, 62.8, 61.0, 55.2, 47.5, 45.9, 41.7, 41.0, 40.8, 40.4. HRMS – found, 880.3700, calc for $C_{54}H_{50}N_5O_7$ $[M+H]^+$, 880.3710.

5.48. Compound 50

Alcohol **49** (0.16 g, 0.18 mmol) was dissolved in dry MeCN (3 mL), evaporated to dryness and redissolved in MeCN (1 mL) and dry DCM (1 mL). Tetrazole (0.013 g, 1 equiv) and then **5** (0.12 g, 2 equiv) in DCM (1 mL) were added. After 0.5 h the reaction was quenched with Et_3N (0.2 mL), concentrated and chromatographed (hexanes – EtOAc – Et_3N, 3:2:0.02) to give a phosphoramidite (0.16 g, ^{31}P NMR δ 146.5 ppm). This material was reacted with **19** (0.080 g, 0.12 mmol 0.8 equiv) using tetrazole (0.020 g, 2 equiv) as the activator according to Method 1 to give **50** (0.12 g, 0.069 mmol, 58% based on **19**) The ^1H and ^{13}C NMR spectra were of little value for characterization of this complex mixture of diastereomers and rotamers. ^{31}P NMR ($CDCl_3$, 202 MHz) δ −1.2, −1.3, −1.5, −2.3. HRMS – found 1731.6045, calc for $C_{93}H_{93}N_{10}O_{20}P_2$ $[M+H]^+$, 1731.6043.

5.49. Compound 51

Hydrolysis according to Method 2 gave alcohol **51** (0.060 g, 0.41 mmol, 61%). ^{31}P NMR δ −1.2, −1.3, −1.4, −2.1. HPLC and LCMS (Kinetex, 60–90% MeCN over 15 min) Rt 5.3 and 5.6 min, (each with base peak 715.2 ($[M+H]^{2+}$), together > 95%).

5.50. Compound 52

Alcohol **51** (0.034 g, 0.024 mmol) and phosphoramidite **17** (0.030 g, 3 equiv) were coupled according to Method 1 using tetrazole (0.005 g, 3 equiv) as the activator. This material was deprotected according to Method 3 and purified on a column of C-18 silica eluted with 10 and 15% MeOH in Et_3NHOAc. Lyophilization gave **54** (4.0 mg, 4.5 mmol, 19%) as a non-

stoichiometric Et_3NH^+ salt. ^1H NMR (D_2O, 500 MHz) δ 8.19 (s, 1H), 7.99 (m, 1H), 7.45 (s, 1H), 5.94 (m, 1H), 4.97 (m, 1H), 4.51 (m, 2H), 4.09 (m, 4H), 3.89 (m, 8H), 3.78 (m, 2H), 3.66 (m, 2H), 3.50 (s, 3H), 2.71 (m, 2H), 2.23 (m, 1H), 1.97 (m, 2H), 1.80 (m, 2H). ^{13}C NMR (D_2O, 126 MHz) δ 166.1, 159.7, 151.7, 150.4, 149.9, 147.1, 144.8, 135.4, 129.0, 117.3, 113.7, 111.4, 84.5, 83.3, 81.7, 72.8, 64.9, 64.6, 63.2, 62.8, 58.3, 46.3, 41.5, 39.6, 32.5. ^{31}P NMR (D_2O, 202 MHz) δ 0.8, 0.2, −0.1. HRMS – found 887.2252, calc for $C_{28}H_{46}N_{10}O_{17}P_3$ $[M+H]^+$, 887.2255. HPLC (Kinetex, 0–30% MeCN over 15 min) R_t 8.57 min, >95%.

5.51. Saporin L3 kinetic studies

Saporin L3 and saporin L3 A14C were expressed as excreted proteins in yeast cultures and purified to near-homogeneity as previously reported [5,22].

Inhibition of SAP was studied in a competitive assay using RNA A10 (5′CGCGAGAGCG3′) as the substrate. Product formation was quantitated in a continuous assay by linking adenine formation to the production of ATP and the action of luciferase to produce light.[4] Inhibitors at varying concentrations were preincubated with enzyme for 10 min at 20 °C in a buffer containing 200 μL of ATPlite (Perkin-Elmer) added into 1 mL of 100 mM Tris-acetate pH 7.7, 2 mM phosphoenolpyruvic acid, 2 mM sodium pyrophosphate, 2 mM 5-phospho-D-ribose 1-diphosphate pentasodium salt, 15 mM $(NH_4)_2SO_4$, 15 mM $(NH_4)_2MoO_4$, 10 mM Mg_2SO_4, 4 units of adenine phosphoribosyl transferase and 8 units of pyruvate phosphate dikinase). The reaction was initiated by adding 100 μM RNA A10. The equilibrium dissociation constants (K_i^*) were calculated by fitting the final, equilibrium reaction rates to equation (1) for competitive inhibition, where v and v_o are steady-state rates in the presence and absence of inhibitor, respectively; K_{A10} is the A10 RNA Michaelis constant[4] and [s] and [I] are the concentrations of the A10 RNA substrate and inhibitor, respectively.

$$v/_{v0} = \frac{K_{A10} + [s]}{K_{A10} + [s] + \frac{K_{A10}[I]}{K_1^*}} \quad (1)$$

In cases where the concentration of inhibitor is < 10-fold the concentration of enzyme, the following correction was applied:

$$I' = I - \left(1 - \frac{v}{v_0}\right) E_t \quad (2)$$

where I′ is the effective inhibitor concentration; I is the concentration of inhibitor used in the assay; v and v_o are initial rates in the presence and absence of inhibitor, respectively; and E_t is the total saporin enzyme concentration used in the assay.

5.52. RNA assays

Yeast tRNA used as substrate was purchased from Invitrogen. Rabbit reticulocyte lysate (untreated) was purchased from Promega (Madison, WI). The rRNA was purified as previously described [5]. The concentration of ribosomal RNA was calculated by the absorbance at 260 nm using the extinction coefficient of 5×10^{-7} cm^{-1} M^{-1}.

6. Protein translation assays

The protein translation inhibited by saporin L3 was studied using a flexi rabbit reticulocyte lysate translation system following the supplier's instructions. The reaction mixtures (50 μL) containing 35 μL of rabbit reticulocyte lysate, 0.5 μL of amino acid mixture, minus leucine, 0.5 μL of amino acid mixture minus methionine, 1.4 μL of 2.5 M KCl, 1 μL of RNasin ribonuclease inhibitor, 1 μL of luciferase-encoding mRNA and different concentrations of saporin L3 A14C ranging from 0.3 to 500 nM were incubated at 30 °C for 90 min. A 10 μL aliquot was measured with a luciferase detection kit following the supplier's protocol in a 96-well plate on a luminometer. The percent translation relative to control was plotted versus the log of SAP A14C concentration and fit to a dose-response curve for the calculation of IC_{50}.

Rescue of translation by inhibitor 2

10 nM SAP A14C was pre-incubated with increasing concentrations of compound **2** for 10 min in 5 μL pH 7.4 buffer. The preincubated samples were incubated with translation reaction (45 μL) at 30 °C for 90 min. Then the luminescence was measured with a luciferase detection kit as described above. A sample with the maximum inhibitor concentration without SAP A14C was used as a control. The percent translation relative to control was plotted versus the log of inhibitor concentration and fit to a dose-response curve for the calculation of EC_{50}.

All molecular structure optimizations and energy and frequency calculations were performed using density functional theory in B3LYP and using a 6–31 g* basis set as implemented in Gaussian 09.[23] The MEPs were calculated with the CUBE program from Gaussian and the files were visualized with GaussView 3.0.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health, USA (Grants CA072444 and GM41916) and by funds provided by the Albert Einstein College of Medicine. Dr. Herbert Wong (Callaghan Innovation) assisted with NMR spectroscopy. Dr. Yinrong Lu (Callaghan Innovation) obtained the high resolution mass spectra.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2016.10.059. These data include MOL files and InChiKeys of the most important compounds described in this article.

Abbreviations

RTA	Ricin A-chain
SAP	saporin L3
MTET	methyltetrazole

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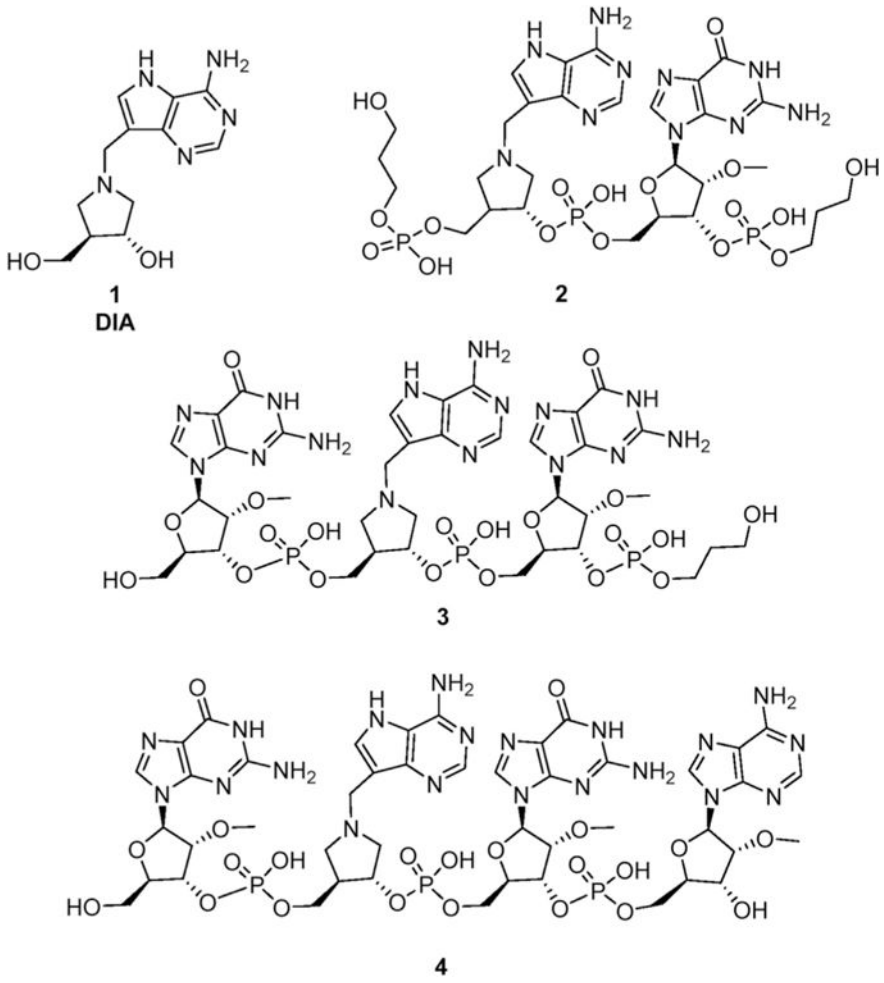


Fig. 1.
Adenosine mimics **1** and some inhibitors of SAP.

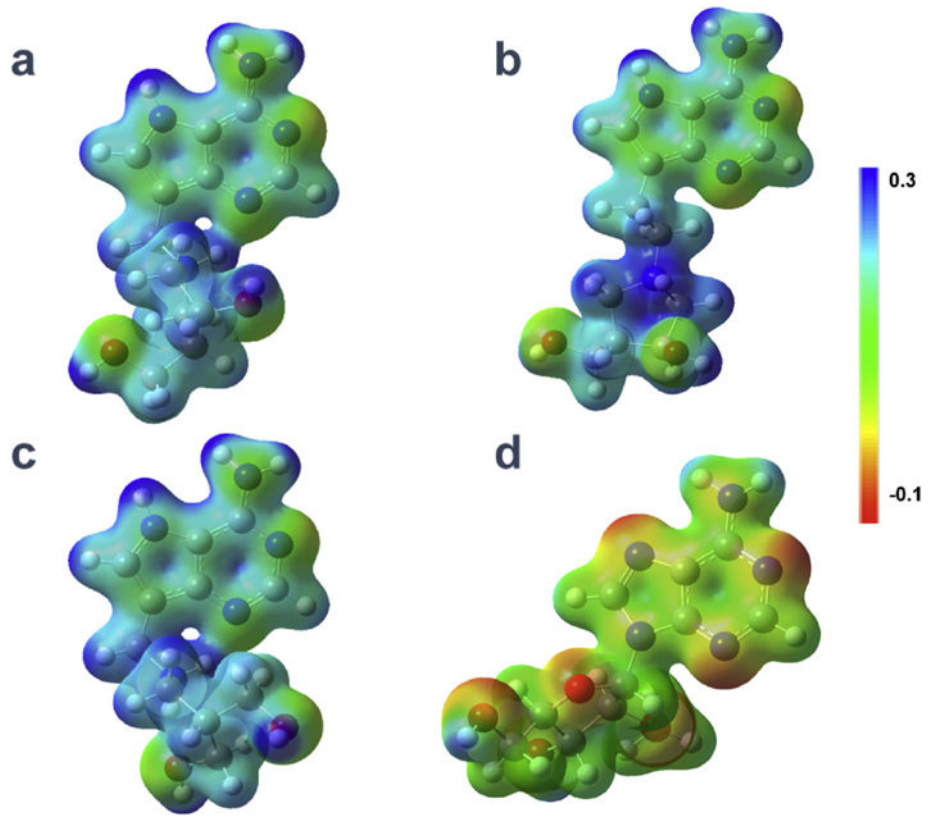


Fig. 2.

Comparison of MEPs for the transition state mimic residues of saporin inhibitors. (a) Compound **2** (PD*p*DIA*p*G*p*PD, K_i^* 3.3 nM, from Yuan et al. [22].); (b) compound **45** (PD*p*DEIA*p*G*p*pd, K_i^* 12.4 nM); (c) compound **54** (PD*p*HomoSer*p*G*p*PD, K_i^* 26.8 nM); and (d) catalytically inactive substrate analogue PD*p*A*p*G*p*PD (K_i^* 16.1 μM).

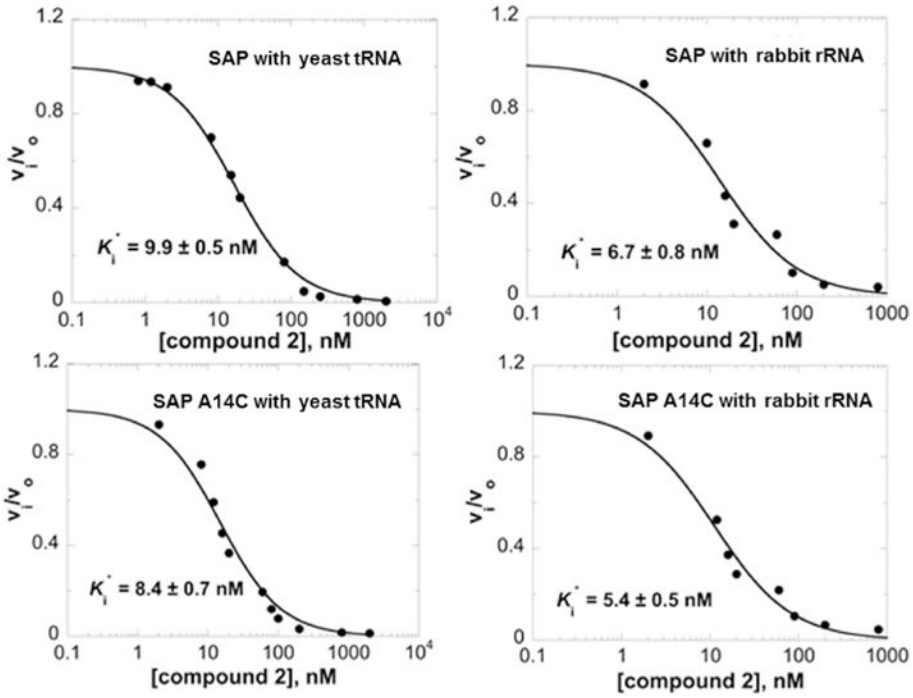


Fig. 3.

Competitive inhibition of SAP as a function of compound **2** with a fixed concentration of isolated rabbit 80S ribosome (100 nM) or yeast tRNA (1.0 mg/mL). Kinetics were measured after a 10 min enzyme-inhibitor preincubation for slow onset binding (K_i^*).

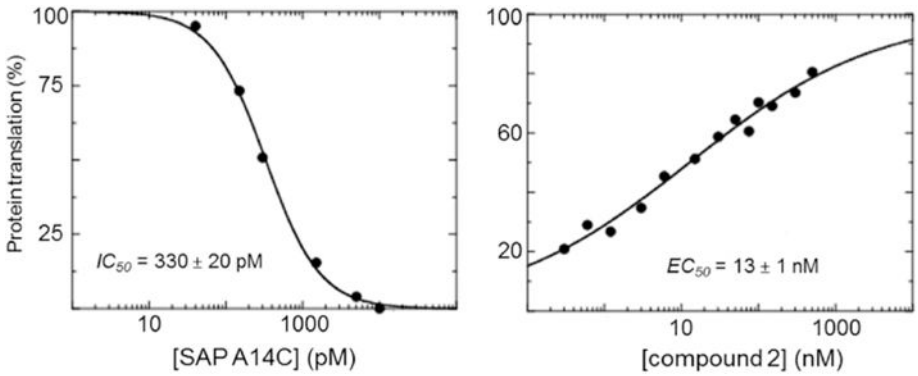
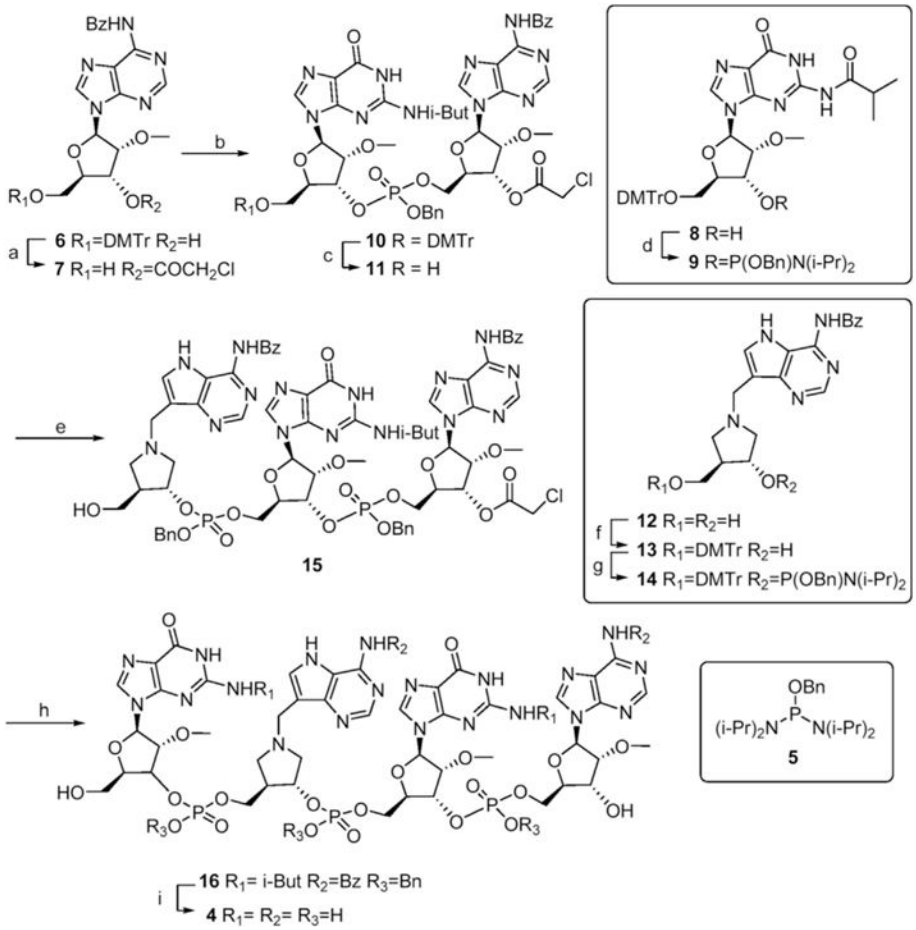


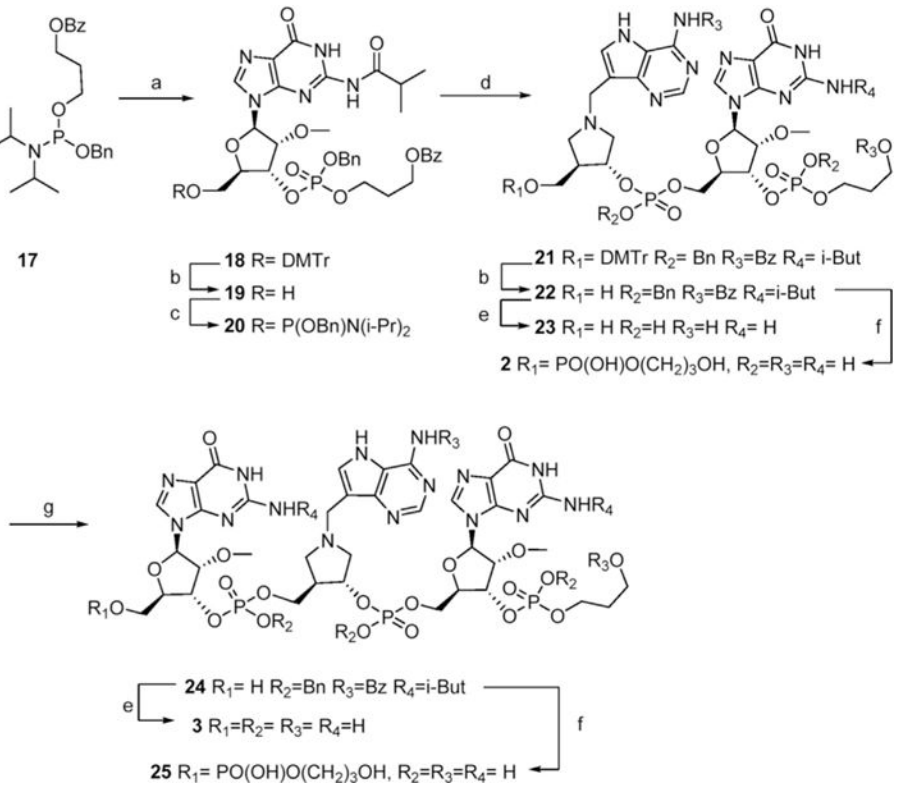
Fig. 4. Inhibition of translation of luciferase mRNA in rabbit reticulocyte extract assays

Left panel, SAP A14C inhibition of luciferase translation in rabbit reticulocyte lysate assay. *Right panel*, Luciferase translation was rescued from 10 nM SAP A14C by compound **2** with an apparent IC_{50} of 13 nM, a 3 nM stoichiometric excess relative to saporin concentration.



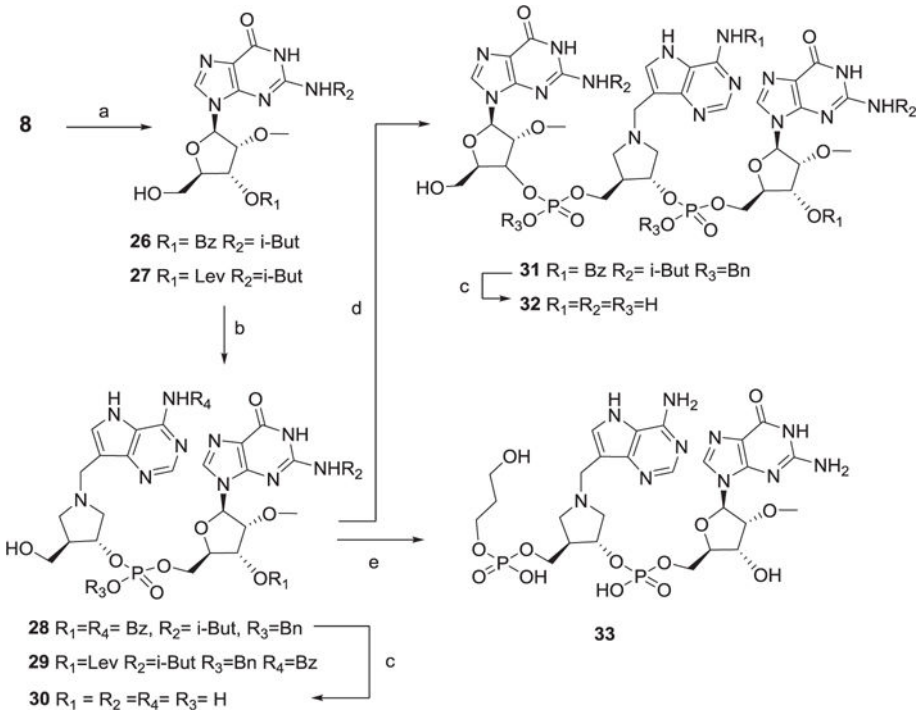
Scheme 1. Synthesis of 4 (G*p*DIA*p*G*p*A)

Reagents and conditions a) $ClCH_2COCl$, pyridine, CH_2Cl_2 then 80% AcOH, 71% b) **9**, MTET, CH_2Cl_2 then *t*-BuOOH, 85% c) 80% AcOH, 72% d) **5**, tetrazole, CH_2Cl_2, 96% e) **14**, MTET, CH_2Cl_2, then *t*-BuOOH, then 80% AcOH, 57% f) DMTrCl, pyridine, 84% g) **5**, MTET, CH_2Cl_2 75% h) **9**, MTET, *t*-BuOOH, then 80% AcOH, 6% i) Pd/C, H_2, then NH_4OH, 40 °C, 39%.



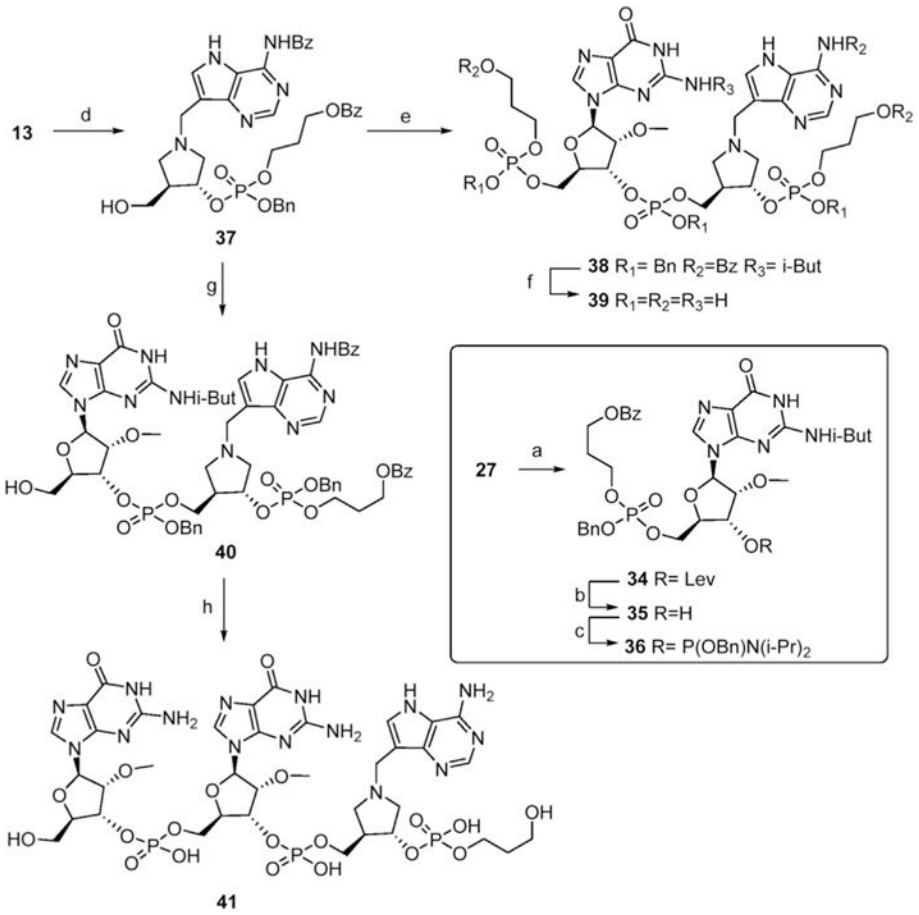
Scheme 2. Synthesis of constructs bearing a DIA – guanosine – propanediol diphosphate (DIA*pG*p*PD*) at the 3′ end

Reagents and conditions a) **8**, DCI, CH_2Cl_2 then *t*-BuOOH, 74% b) HCl-MeOH, 100% of **19**, 75% of **22** c) **5**, tetrazole, CH_2Cl_2, 85% d) **13**, tetrazole, CH_2Cl_2 then *t*-BuOOH, 42% e) Pd/C, H_2, then NH_4OH, 50 °C, 24 h, 52% of **23**, 29% of **3** f) **17**, tetrazole, CH_2Cl_2 then *t*-BuOOH then Pd/C, H_2, then NH_4OH, 50 °C, 24 h, 29% of **2**, 7% of **25** g) **9**, tetrazole, CH_2Cl_2 then *t*-BuOOH then HCl-MeOH, 26%.



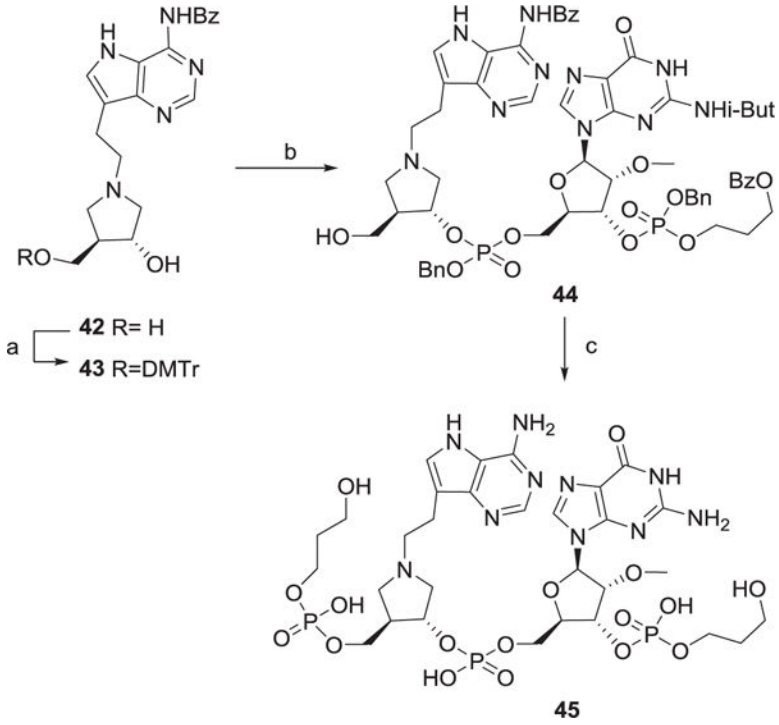
Scheme 3. Stepwise synthesis of 30, 31 and 32

Reagents and conditions a) BzCl, pyridine, then 80% AcOH (for **26**, 63%), levulinic acid, EDCI, DMAP, THF, then HCl-MeOH (for **27**, 90%) b) **14**, tetrazole, CH_2Cl_2 then *t*-BuOOH, then for **28**, 80% AcOH 95%, for **29** HCl-MeOH, 53% c) $Pd(OH)_2$-C then NH_4OH, 50 °C, 24 h, for **30** 31%, for **32** 23% d) **9**, tetrazole, CH_2Cl_2, then *t*-BuOOH, then 80% AcOH, 66% e) **17**, tetrazole, CH_2Cl_2, then *t*-BuOOH, then H_2, Pd-C then NH_4OH, 50 °C 24 h, 11%.



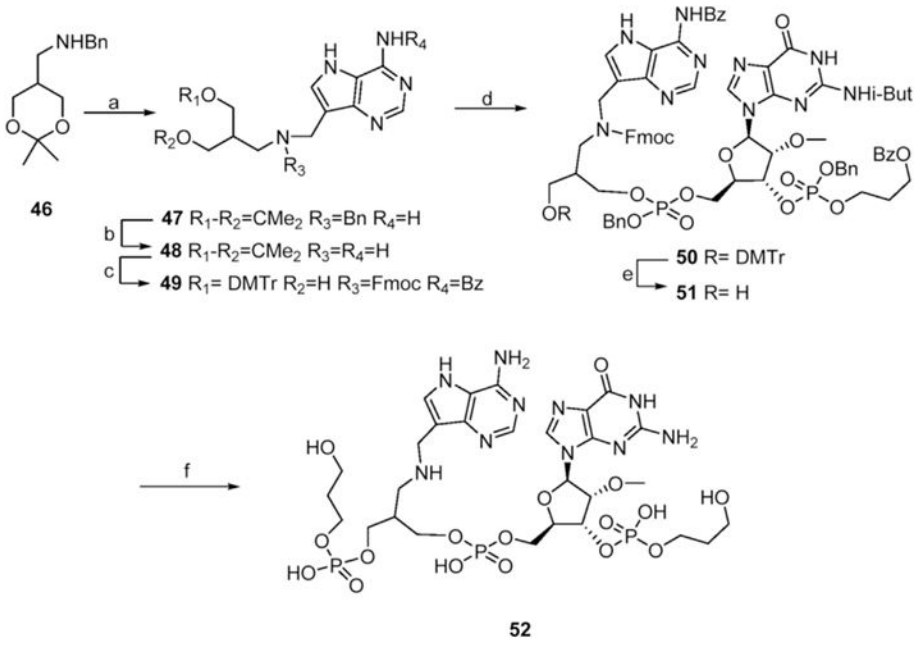
Scheme 4. Convergent synthesis of 39 and synthesis of 41

Reagents and Conditions a) **17**, DCI, CH_2Cl_2 then *t*-BuOOH, 50% b) N_2H_4-H_2O, MeOH, 5 h, 70% c) **5**, DCI, CH_2Cl_2, 86% d) **17**, tetrazole, CH_2Cl_2 then *t*-BuOOH then HCl-MeOH,, 66% e) **36**, tetrazole, CH_2Cl_2 then *t*-BuOOH, 32% f) Pd-C, H_2 then NH_4OH, 50 °C, 24 h, 43% g) **9**, tetrazole, CH_2Cl_2 then *t*-BuOOH then HCl-MeOH, 50% h) **9**, tetrazole, CH_2Cl_2 then *t*-BuOOH then HCl-MeOH, then Pd-C, H_2 then NH_4OH, 50°C 24 h.



Scheme 5. Synthesis of DIEA compound 45

Reagents and conditions a) DMTrCl, pyridine, 33% b) **20**, tetrazole, CH_2Cl_2 then *t*-BuOOH, then HCl-MeOH, 40% c) **17**, tetrazole, CH_2Cl_2 then *t*-BuOOH, then H_2, Pd-C, then NH_4OH, 50°C, 24 h, 9%.



Scheme 6. Synthesis of Homoserinol construct 52

Reagents and conditions a) 9-deazaadenine, CH_2O, EtOH, 60 °C, 52% b) H_2, $Pd(OH)_2$–C, 93% c) FmocCl, $NaHCO_3$, MeOH, then BzCl, pyridine then THF – AcOH, 2:1 then DMTrCl, pyridine, 35%. d) **5**, tetrazole, CH_2Cl_2 then **19**, tetrazole, CH_2Cl_2 then, *t*-BuOOH, 58% e) HCl-MeOH, 61% f) **17**, tetrazole, CH_2Cl_2 then *t*-BuOOH, 19% then H_2, Pd – C then NH_4OH, 50 °C, 19%.

Table 1

Inhibition constants for SAP inhibitors.

Compound	Construct	Inhibition constant K_i^* (nM)[a]
2	PD*p*DIA*p*G*p*PD	3.3 ± 0.2
3	G*p*DIA*p*G*p*PD	10.5 ± 0.9
4	G*p*DIA*p*G*p*A	6.1 ± 0.3
23	DIA*p*G*p*PD	10,700 ± 800
25	PD*p*G*p*DIA*p*G*p*PD	7.4 ± 0.4
30	DIA*p*G	21,300 ± 800
32	G*p*DIA*p*G	33.0 ± 1.7
33	PD*p*DIA*p*G	19.3 ± 1.7
39	PD*p*G*p*DIA*p*PD	188 ± 12
41	G*p*G*p*DIA*p*PD	212 ± 17
45	PD*p*DEIA*p*G*p*PD	12.4 ± 0.9
52	PD*p*HomoSer*p*G*p*PD	26.8 ± 0.3
	PD*p*Ser*p*G*p*PD	43.2 ± 1.1[b]
	PD*p*A*p*G*p*PD (substrate analogue)	16,000 ± 1500

[a] K_i^* is the dissociation constant K_d for the inhibitor interactions with SAP following slow–onset inhibition.

[b] Values taken from Schramm et al. [19].