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New phosphoramidites of RNA cleaving trisbenzimidazole catalysts: improved compatibility with oligonucleotide solid phase synthesis

Dennis Krug, Konstantinos Chionis, Jonka Tepe, Karyna Stoliarchuk, Janina Möckel, Karin Göbel, and Michael W. Göbel*

Institute for Organic Chemistry and Chemical Biology, Goethe University Frankfurt, Max-von-Laue-Strasse 7, D-60438 Frankfurt am Main, Germany
Email: m.goebel@chemie.uni-frankfurt.de

This article is dedicated to Professor Hans-Günther (Hagga) Schmalz in celebration of his outstanding career and on the occasion of his retirement

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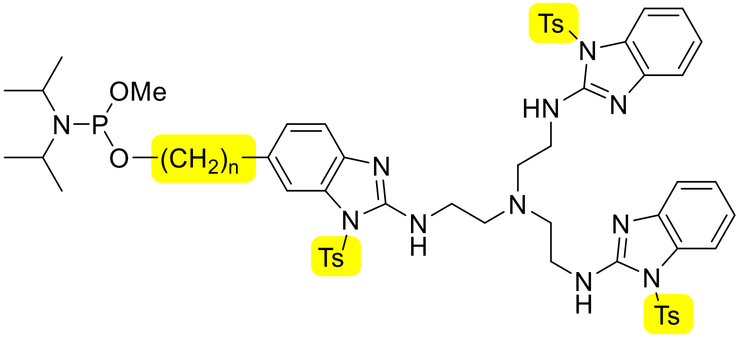
Abstract

Phosphoramidite building blocks of trisbenzimidazole catalysts can be attached in high yield to the 5' end of oligonucleotides and convert them into site-specific RNA cleavers. Previous amidites, however, are not fully compatible with all types of DNA synthesizers. In this study we have tested several linker structures and protective groups. Tosyl protected compounds with alkyl linkers no longer show the former problems and can be coupled like standard DNA amidites. Although deprotection conditions are harsher for tosyl than for Boc, they are tolerated by the majority of nucleosides. If required, it is also possible to replace the tosyl groups with Boc.



Keywords: Artificial ribonuclease, guanidine analog, protective group, metal-free catalyst, Sonogashira reaction, Suzuki-Miyaura reaction

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Introduction

RNA cleaving conjugates of oligonucleotides and tris(2-aminobenzimidazoles) are versatile tools to destroy target RNAs [1] or to cut them specifically for subsequent analysis.[2] If only few of such conjugates are required, a manual coupling protocol with phosphoramidite **1** (Figure 1) will be the method of choice.[3] However, for the production of larger numbers, the catalyst attachment should be executed automatically by the oligonucleotide synthesizer. Unfortunately, pre-mixing of phosphoramidite **1** and tetrazole derivatives in the absence of nucleophiles leads to permanent deactivation of the reagent: the protonated amidite seems to react with a nucleophilic site within structure **1**. Plausible candidates are the carboxamide linker and the nitrogen atoms of the heterocyclic guanidines. Depending on the synthesizer type, automated coupling with reagent **1** can proceed well if the activated amidite is immediately pumped into the synthesis column.[2] Any delay after activation must be avoided and is deleterious for the coupling yield. Although the problem can be controlled, analogs of amidite **1** without such limitations are desirable. In a study to optimize side chain structures and protecting groups, our focus therefore was on carboxamide-free alkyne- and alkyl-linkers. In this study we present the synthesis and evaluation of the new trisbenzimidazole phosphoramidites **2** – **6** (Figure 1).

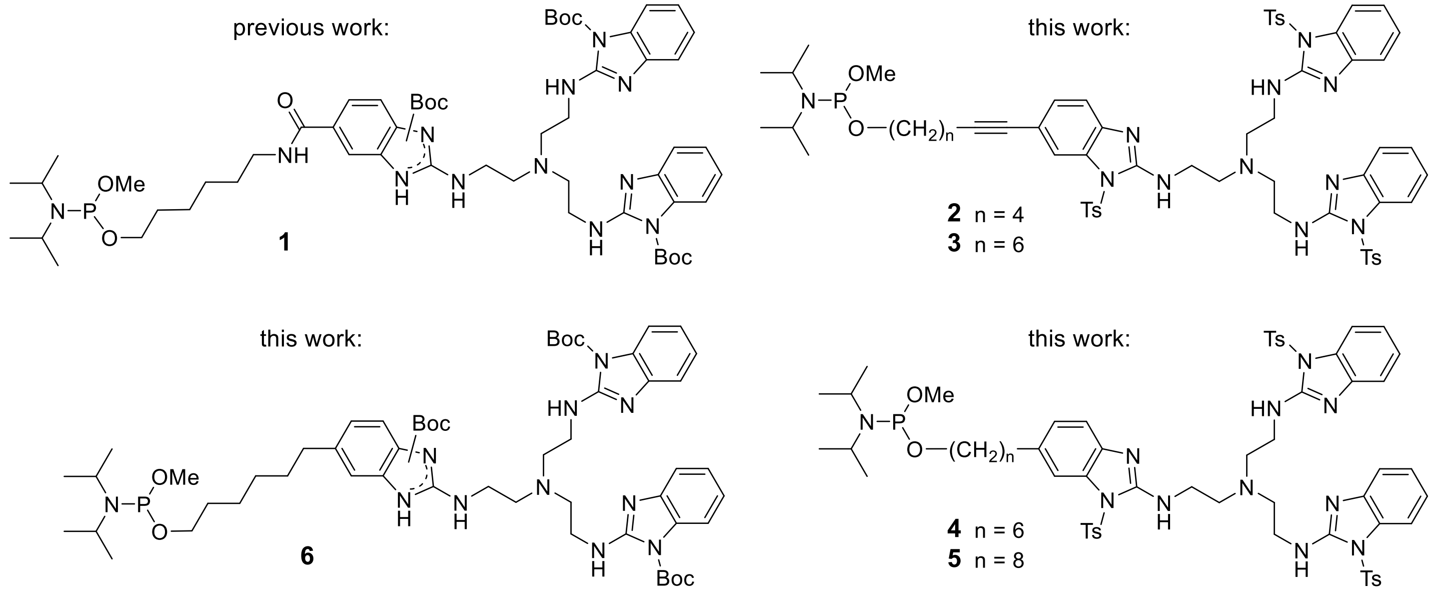


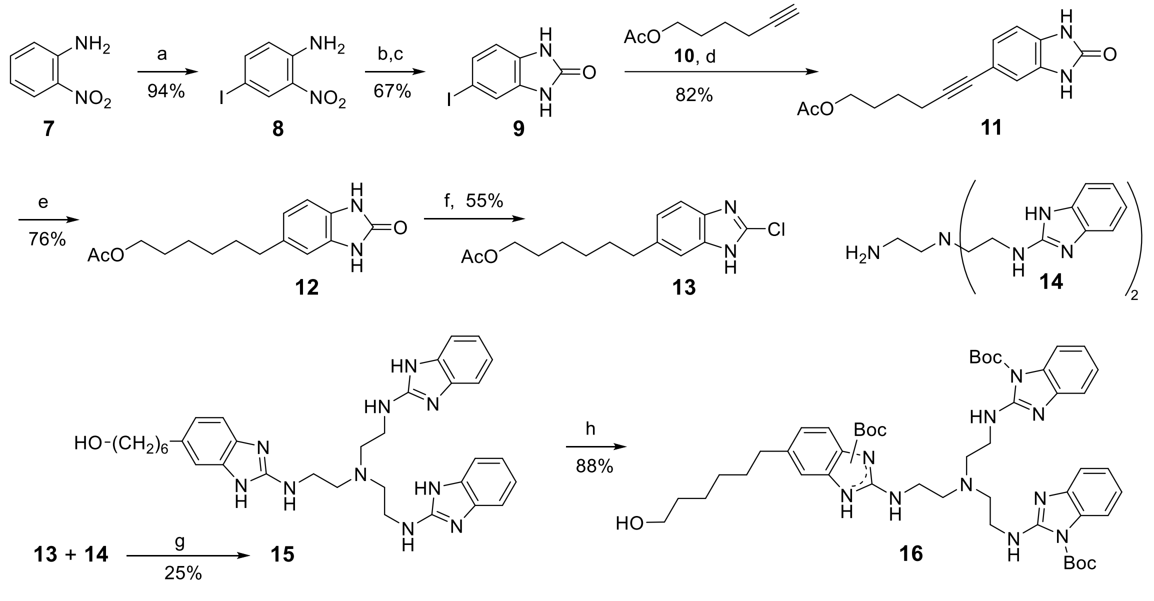
Figure 1. Phosphoramidite building blocks of RNA-cleaving trisbenzimidazole catalysts.

Results and Discussion

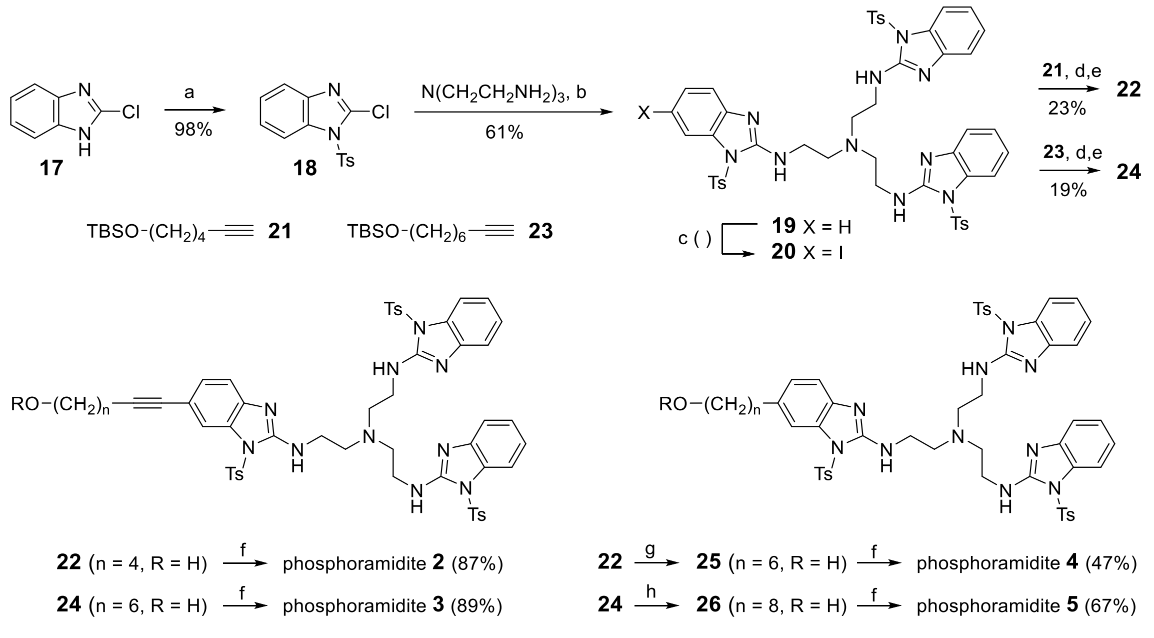
Synthesis of new phosphoramidites

A first step-by-step synthesis of amidite **6** started from 5-iodobenzimidazolone **9**, prepared from 2-nitroaniline **7** in fair yield and high purity (Scheme 1).[4] The side chain was then attached by Sonogashira coupling with alkyne **10** (82% of **11**). Subsequent transfer hydrogenation with Pd/C and Et$_3$SiH reduced the triple bond (**12**, 76%). Chlorination of benzimidazolone **12** with POCl$_3$ in acetonitrile afforded compound **13** (55%). The ester moiety was necessary in this step to protect the side chain oxygen. Finally, the mixed trisbenzimidazole **15** was obtained in a microwave assisted reaction of **13** and TREN derivate **14** [5] followed by ester hydrolysis (25%). Boc protection

(**16**, 88%) and phosphitylation converted alcohol **15** into the phosphoramidite **6**. However, larger quantities of compound **6** were prepared following a more efficient synthetic route towards compound **16** (see below).



Scheme 1. Stepwise synthesis of the precursor **16** of phosphoramidite **6**. a) ICl, HOAc, 94%; b) $SnCl_2 \cdot 2\ H_2O$, HCl; c) 1,1-carbonyldiimidazole, THF, 67% from **8**; d) **9** + **10**, $(Ph_3P)_4Pd$, CuI, Et_3N, DMF, 82%; e) 10% Pd/C, Et_3SiH, MeOH, 76%; f) $POCl_3$, CH_3CN, microwave heating, 55%; g) 1. **13** + **14**, ethylene glycol, microwave heating; 2. LiOH, THF/water, microwave heating, 25%; h) Boc_2O, DIPEA, DMF, 88%.



Scheme 2. Synthesis of phosphoramidites **2** – **5** via statistical iodination of trisbenzimidazole **19**. a) TsCl, Et_3N, DCM, 98%; b) TREN, Cs_2CO_3, C_6H_6, 61%; c) 1 eq. NIS, HOAc, not purified; d) **21** (or **23**), $(Ph_3P)_2PdCl_2$, CuI, Et_3N, DMF, not purified; e) HOAc, THF, H_2O, 23% (19%) from **19**; f) methyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 47% - 89%; g) Raney-Ni, H_2, THF, EtOH, 43% ; h) Pd/C, H_2, THF, EtOH, 99%.

In phosphoramidites **2** – **5**, we have replaced the Boc groups by tosyl. This change can shorten the synthesis significantly (Scheme 2). After oligonucleotide assembly, the removal of the tosyl groups needs more vigorous conditions compared to the removal of Boc but the procedure is still compatible with the majority of nucleotides. 2-Chloro-1-tosylbenzimidazole **18**,[6] obtained from commercial **17** in high yield, is a bifunctional electrophile. In polar solvents, it preferentially transfers the sulfonyl group.

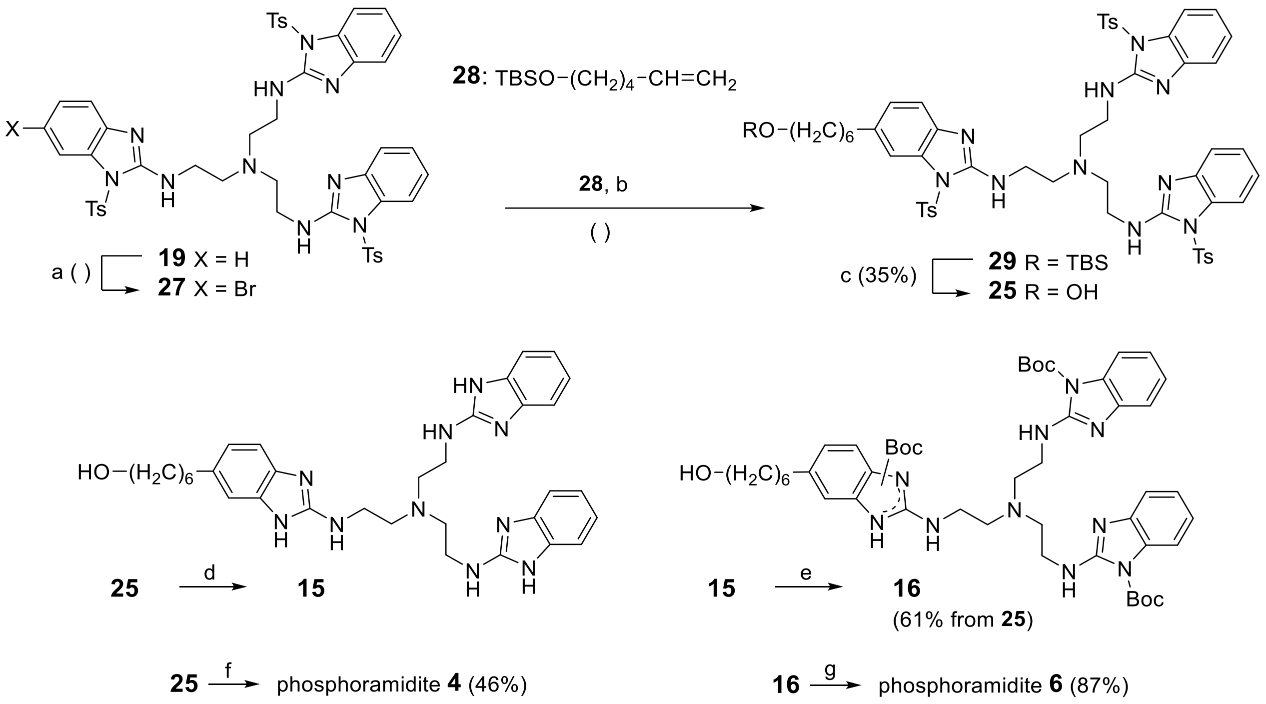
Thus, reaction of **18** and TREN (tris(2-aminoethyl)amine) in DMF leads to pertosylated TREN and compound **17** as sole products. Nonpolar solvents like benzene are required to obtain the trisbenzimidazole **19**. With powdered Cs_2CO_3 as base 61% of **19** were obtained. In toluene as solvent and K_2CO_3 as base the yield was significantly reduced. We then treated compound **19** with 1 equiv. of NIS in acetic acid to achieve a statistical mono-iodination para to the nontosylated ring nitrogen atoms of the benzimidazoles.[7,8] By submitting the crude product, containing compound **20**, to Sonogashira coupling with alkyne **21**, a mixture of trisbenzimidazoles resulted containing none, one or two alkyne side chains. After desilylation, a separation by chromatography yielded 23% of alcohol **22** over three steps from compound **19**. Alcohol **22** was then converted into phosphoramidite **2** (87%).

In the same way, Sonogashira coupling of the C_8 alkyne **23** and crude iodo trisbenzimidazole **20** followed by desilylation and chromatography produced alcohol **24** (19%), the precursor of phosphoramidite **3** (89%). Trisbenzimidazole alcohols with saturated side chains were produced by catalytic hydrogenation of alkynes **22** and **24**. In the first case, Raney-Ni was used as catalyst. This reaction suffered from irreversible product adsorption on the catalyst lowering the yield of **25** to 43%. In contrast, hydrogenation of **24** with slightly increased amounts of Pd on carbon proceeded almost quantitatively. Alcohols **25** and **26** could be converted into the phosphoramidites **4** and **5** (47% and 67%).

In RNA cleavage experiments, oligonucleotide conjugates prepared from phosphoramidite **4** showed the best performance (see below). We therefore searched for an improved synthetic pathway starting from trisbenzimidazole **19** (Scheme 3). After statistical reaction with 1 eq. of NBS, a Suzuki-Miyaura reaction was conducted with the crude bromination product **27** and an alkyl borane obtained by hydroboration of compound **28** with 9-BBN.[9] This reaction failed with $(Ph_3P)_2PdCl_2$, $(Ph_3P)_4Pd$ or combinations of Pd salts with SPhos. In remarkable contrast, fast coupling occurred when the Buchwald precatalyst Pd SPhos G3 was applied.[10,11] Product **29** was in part purified by chromatography.

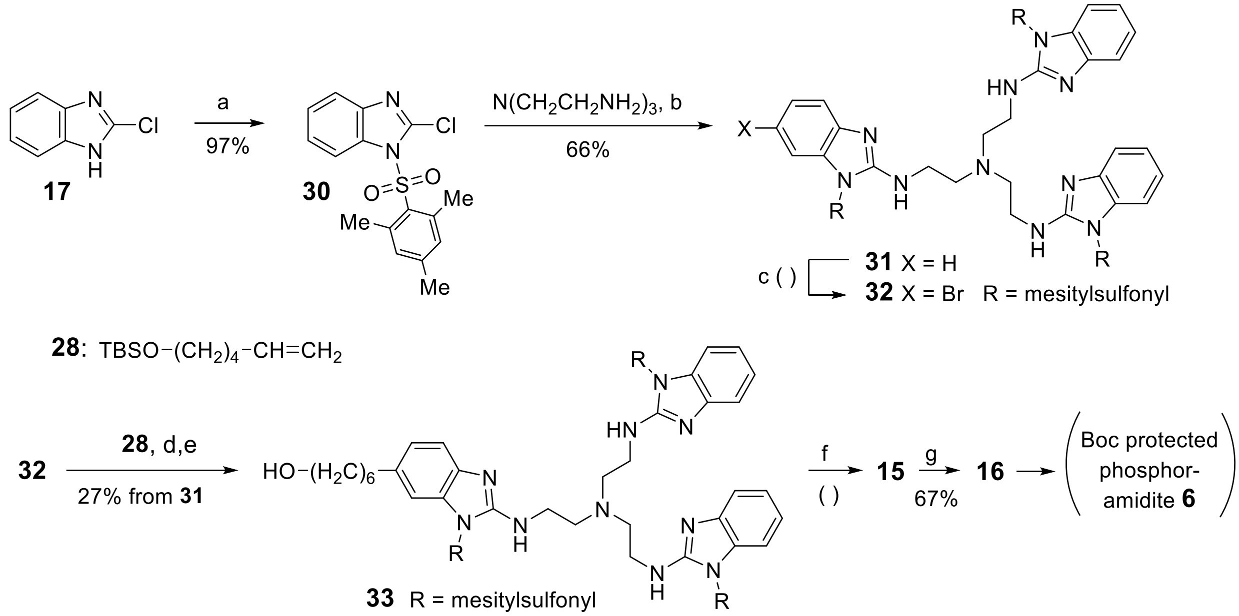
After desilylation of **29**, a second chromatography provided pure alcohol **25** on gram scale in 35% total yield from compound **19**. To appreciate this result, one has to consider that the bromination yield for statistical reasons cannot be much above 40%. Alcohol **25** was converted into phosphoramidite **4** as described above. Alternatively, the tosyl groups could be removed with aqueous NaOH. The resulting trisbenzimidazole **15** finally was reprotected with Boc_2O to give compound **16** in 61% yield, the precursor of phosphoramidite **6** (87%).

Compared to polar unprotected trisbenzimidazoles, the handling of the tosylated derivative **19** during extraction and chromatography is much more convenient. We therefore tested - in addition to tosyl, mesyl and 2,4,6-trimethylbenzenesulfonyl as potential protective groups. A mesylated variant of phosphoramidite **4** could be prepared by analogous methods and behaved well in the coupling step. The deprotection of the resulting conjugates, however, required harsh conditions and was thus abandoned.



Scheme 3. Optimized synthesis of phosphoramidites **4** and **6** via statistical bromination of trisbenzimidazole **19**. a) 1 eq. NBS, HOAc, not purified; b) **28**, 9-BBN, THF, then addition to **27**, Pd SPhos G3, SPhos, K_3PO_4, THF + H_2O, not purified; c) HOAc, THF, H_2O, 35% from **19**; d) NaOH, THF, MeOH, H_2O, not purified; e) Boc_2O, Et_3N, DMF, 61% from **25**; f) methyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, 4,5-dicyanoimidazole, DCM, 46%; g) methyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 87%.

The 2,4,6-trimethylbenzenesulfonyl group is again too stable for direct use in phosphoramidites. But due to increased steric hindrance and lipophilicity, it showed distinct advantages in the synthesis of trisbenzimidazoles. Starting from compound **17**, the sulfonylation yield was almost quantitative. As in the case of **18**, compound **30** is a bifunctional electrophile that may transfer the benzimidazole or the sulfonyl group to amino nucleophiles. The mesityl ring, however, retarded the sulfonyl transfer and led to improved yields of trisbenzimidazole **31** (66%). Statistical bromination and Suzuki-Miyaura reactions then attached the silylated side chain. While the chromatography of the tosylated compound **29** did not achieve full resolution, the Suzuki-Miyaura product of the crude bromination product **32** could be well separated into fractions containing one (33%), two or three silylated side chains. Acid-induced desilylation finally yielded compound **33** (83%; 27% from **31** together with recovered starting material **31**). To convert compound **33** into the Boc analog **16**, we removed the sulfonyl groups with NaOH at 60 °C for 18 min and treated the crude trisbenzimidazole **15** directly with Boc_2O.[3] Pure compound **16** was then obtained after chromatography in 67% yield.



Scheme 4. Synthesis of trisbenzimidazole **16** via mesitylsulfonylated intermediates. a) 2,4,6-trimethylbenzenesulfonylchloride, Et_3N, DCM, 97%; b) TREN, Cs_2CO_3, C_6H_6, 66%; c) 1 eq. NBS, HOAc, not purified; d) **28**, 9-BBN, THF, then addition to **32**, Pd SPhos G3, SPhos, K_3PO_4, THF + H_2O; e) HOAc, THF, H_2O, 27% from **31**; f) NaOH, THF + MeOH + H_2O; g) Boc_2O, DIPEA, DMF, 67% from **33**.

Synthesis of conjugates

The manual coupling protocol previously used for phosphoramidite **1** also worked well for all new analogs **2** – **6** described here.[2] Conjugates **34** – **38** (Figure 2) have been prepared in this way. Removal of Boc from conjugate **34** required incubation in 25% NH_4OH for 16 h at 55 °C in a closed vial.[3] In contrast, only partial removal of tosyl under such conditions was observed with conjugates **35** – **40** (Figure S3). Therefore, AMA treatment (conc. ammonia / 40% aqueous methylamine) at 65 °C for 3-5 h or at 45 °C for 20 h is required for complete deprotection (Figures S1-S7). Such forcing conditions, however, are not compatible with benzoyl protected cytidine derivatives.

The expedite synthesizer used in our lab routinely mixes phosphoramidites and activator before pumping the reagents into the synthesis column. As mentioned above, this causes side reactions with phosphoramidite **1** and coupling yields are close to zero. In contrast, conjugate yields rose to about 25% with the Boc-protected analog **6** and to high values with the tosyl-protected amidite **4**. Although the replacement of the carboxamide linker is beneficial, the higher electron withdrawing effect of the sulfonyl groups seems to be crucial for stabilizing the amidite building blocks. There is also a technical solution of the problem: a minor change in the synthesizer protocol [2] has allowed us to couple even amidite **1** with fair results. Amidites **4** and **6** behave like ordinary DNA amidites under such conditions.

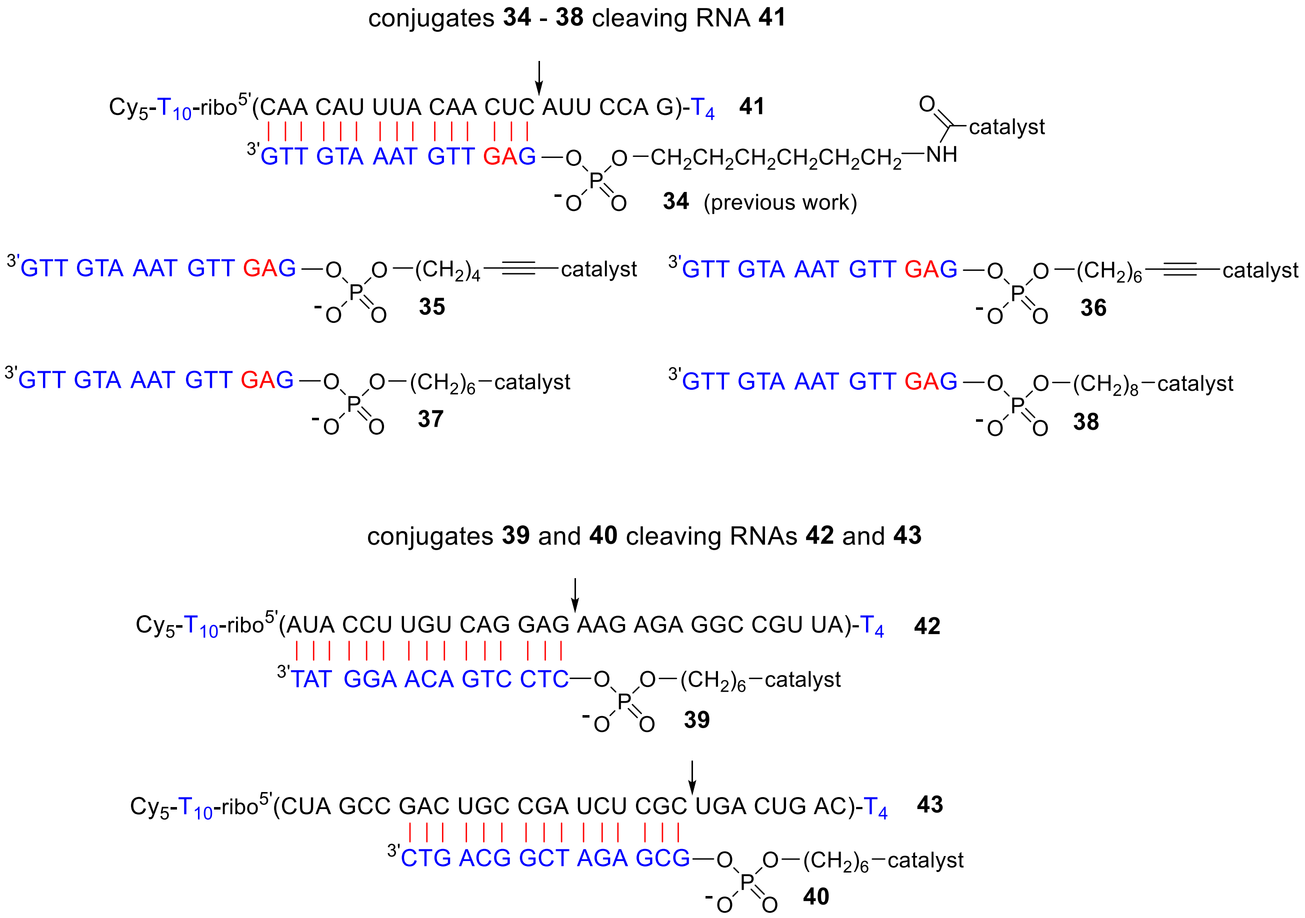


Figure 2. RNA cleaving conjugates **35** – **40** prepared from phosphoramidites **2** – **6** and RNA substrates **41** – **43**. DNA nucleotides are shown in blue, RNA in black and LNA in red. RNA cleavage by conjugate **34**: see ref 3. The main cleavage sites in each RNA are marked with black arrows.

Cleavage experiments

The RNA substrates **41** – **43** are 5′ labeled with a fluorescent dye (Figure 2) to detect and quantify fragments after separation by gel electrophoresis in an ALFexpress DNA sequencer.[12] The RNA part is surrounded by DNA nucleotides to improve the resolution of all possible fragments. Cleavage by intramolecular nucleophilic attack at phosphates requires the 2′-OH groups of ribonucleotides. Thus, partial hydroxide induced degradation of substrate **41** – containing 22 ribonucleotides – generated 22 well separated fragment peaks (Figure 3). Cleavage by oligonucleotide-trisbenzimidazole conjugates strictly depends on base pairing and guided catalyst-substrate proximity.[12] Normally it occurs at the end of the substrate-conjugate duplex. The reaction mechanism involves general acid/base catalysis leading to 2′,3′ cyclic phosphates. Conjugates **34** – **38** all hybridize with the first 15 ribonucleotides of substrate **41** (Figure 2). Thus, cleavage should occur between ribonucleotides 15 and 16. As expected, the experiment with conjugate **37** (Figure 3) showed a dominating peak which coincides with fragment 15 of the base ladder and makes up almost 90% of all reaction products (Table 1).

To compensate the low GC content in the 15mer binding site of RNA **41**, two LNA modifications (locked nucleic acid, shown in red) were introduced in conjugates **34** – **38**. This had been shown previously to stabilize the resulting duplex significantly.[3] Reaction rates were then measured under saturation conditions when all binding sites were occupied with conjugate (150 nM **41**, 1.2 µM **37**, Figures S24, S28). Kinetics followed a first-order rate law with $k_{obs} = 0.12\ h^{-1}$ ($t_{½} = 5.8$ h, Table 1). In terms of rates and product pattern, conjugate **34** containing the carboxamide linker revealed very similar results.[3] Elongation of the alkyl linker by two CH_2 groups in conjugate **38** again caused no significant difference. Slower cleavage, however, was observed with alkyne conjugates **35** and **36** (Table 1).

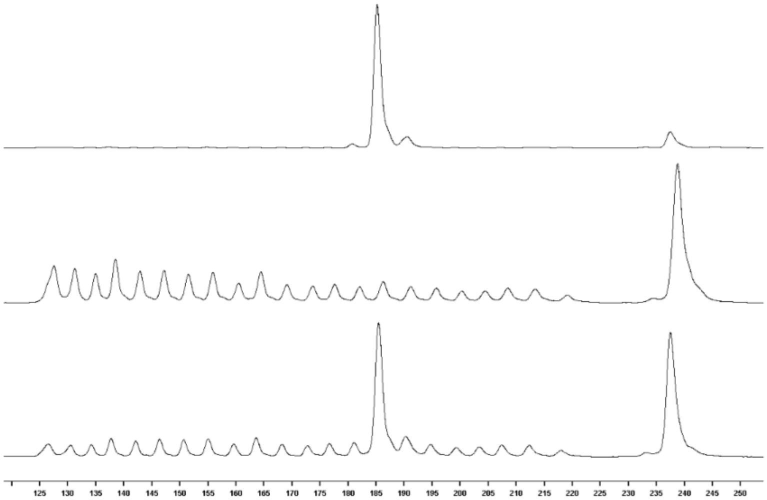


Figure 3. Cleavage of RNA **41** by conjugate **37**, analyzed by gel electrophoresis on an ALFexpress DNA sequencer. Before incubation, the full-length strand of **41** forms a single peak appearing at 238 min (not shown). Top: RNA **41** (150 nM) after incubation with **37** (1.2 µM) for 18 h (pH 8.0, 37 °C) forms a major cleavage fragment (186 min; only dye-labeled fragments are visible). Center: base ladder of the 22mer RNA **41** showing 22 fragment peaks. Bottom: hydrolysis ladder of **41** spiked with the cleavage mixture. The main fragment coincides with fragment 15 from the hydrolysis ladder.

Table 1

entry	conjugate	**34**[a]	**35**	**36**	**37**	**38**	**39**	**40**
1	target RNA	**41**	**41**	**41**	**41**	**41**	**42**	**43**
2	k_{obs} [b] [h^{-1}]	0.107	0.067	0.074	0.12	0.11	0.054	0.092
3	$t_{½}$ [b] [h]	6.5	10.3	9.4	5.8	6.3	12.8	7.5
4	main fragment [c]	88.3%	87.4%	88.4%	89.7%	88.7%	71.7%	79.0%

a) see ref. 3; b) determined under saturation conditions, experimental error ±10%; c) area of the main peak divided by the sum of all fragment peaks.

Each of the conjugates **37**, **39** and **40** was synthesized twice, using either the tosylated phosphoramidite **4** or the Boc analog **6** (Figures S19-S22). Identical cleavage patterns and kinetics were found, independent from the amidite used. In the case of conjugate **39** a substrate half-life of 12.8 h was measured for RNA **42** in accord with data published previously for a similar conjugate prepared from phosphoramidite **1** ($t_{½}$ of RNA **42**: 12.4 h).[12] Conjugate **40** finally targeted RNA **43** (Figure 2, Table 1). Cleavage kinetics of conjugates **37**, **38**, **39**, and **40** have been also repeated in the presence of 1 mM EDTA without causing changes in rates or fragment patterns. Accordingly, the influence of metal ion contaminations can be excluded.

RNA cleavage by nucleophilic attack of 2′ hydroxy groups implies the formation of 2′,3′ cyclic phosphates as the primary products. Recently, we could demonstrate by mass spectrometry that fragments produced by trisbenzimidazole catalysts indeed end with 2′,3′ cyclic phosphates exclusively. In that study, the cleavage fragments dissociated rapidly from the catalyst conjugate and thus escaped from the trisbenzimidazole part.[13] In contrast, 5’ conjugates such as compounds **34** – **40** do not reduce the number of conjugate-substrate base pairs upon cleavage. Dissociation of fragments therefore is slow and the cyclic phosphates remain in the neighborhood of the catalyst. Secondary cleavage to form 2’ or 3’ monophosphates therefore can be expected. Mass spectrometric fragment analysis of RNA **41** after incubation with conjugate **37** supported this view: the monophosphates clearly outweighed the cyclic phosphates (Figure S23).

Conclusions

The polar nature of unprotected trisbenzimidazoles and the limited solubility in many solvents complicate the workup and chromatographic purification in several steps of our previous syntheses. The reaction of TREN with *N*-sulfonylated 2-chlorobenzimidazoles, in contrast, produces highly soluble intermediates that can be purified by chromatography more easily. Attaching alkyl side chains to the tosylated symmetric trisbenzimidazole **19** can be achieved by partial bromination, Suzuki-Miyaura coupling and desilylation of the alkyl linker in 35% total yield of **25**. This compound is formed as a single isomer whereas Boc derivative **16** consists of two constitutional isomers. Increasing the lipophilicity by replacing tosyl with mesitylsulfonyl groups (compound **31**) improves further the chromatographic behavior.

In contrast to phosphoramidite **1**, the new compound **4** reacts in automated oligonucleotide synthesis equally efficient as standard DNA amidites, supporting the view that nucleophilic sites in **1** are responsible for side reactions. Although the removal of tosyl requires harsher conditions compared to Boc, amidite **4** is compatible with the majority of oligonucleotides. However, for strands containing benzoyl protected cytidines, the Boc protected amidite **6** in combination with a modified synthesizer protocol is preferable. Amidite **6** can be prepared from compound **25** (61%) or **33** (67%) by base induced removal of sulfonyl and reprotection with Boc_2O. In terms of cleavage pattern and kinetics, the replacement of the carboxamide side chain (amidite **1**) by an C_6 alkyl linker (amidites **4** and **6**) has no significant effect.

Experimental Section

General: All chemicals were reagent grade and used as purchased. Dry solvents were purchased in a septum bottle with molecular sieves from Thermo Fischer and used without further purification. A Discover 2.0 microwave synthesizer (CEM, Kamp-Lintfort, Germany) was used to conduct some reactions at elevated temperatures. The reactions were monitored by TLC using ALUGRAM Xtra SIL G/UV_{254} aluminum sheets (Macherey-Nagel). Compounds were visualized by UV light (254 and 366 nm) or by staining with ninhydrin solution. Column chromatography was carried out on silica gel 60 (0.04–0.063 mm, Macherey-Nagel). Proton, carbon, silicon and phosphorus nuclear magnetic resonance spectra (^1H NMR, ^{13}C NMR, ^{29}Si and ^{31}P NMR) were recorded at 300 K with the following spectrometers by Bruker (Karlsruhe, Germany):

	$\nu_L(^1H)$ [MHz]	$\nu_L(^{13}C)$ [MHz]	$\nu_L(^{29}Si)$ [MHz]	$\nu_L(^{31}P)$ [MHz]
AV 400	400	101	80	162
AV 500	500	126	-	-
DRX 600	600	151	-	-

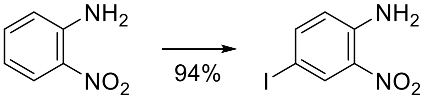
Chemical shifts are reported in parts per million (δ scale) and internally referenced to the resonances of the solvent for ^1H- and ^{13}C-NMR:

	$CDCl_3$	DMSO-*d*$_6$	C_6D_6
δ [ppm] ^1H	7.26 (s)	2.5 (quin)	7.16 (s)
δ [ppm] ^{13}C	77.16 (t)	39.52 (sept)	128.06 (t)

The data are represented, as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets), coupling constants in Hz and integration. The IR spectra were recorded with a Jasco FT/IR-420 spectrometer (Jasco Germany GmbH, Pfungstadt, Germany) equipped with an ATR unit. ESI-MS spectra were obtained on a *Thermo Fisher single quadrupole electrospray ionisation MS*. HRMS spectra were recorded on a *Bruker microTOF-II*. Oligonucleotides and conjugates were characterized by LC-MS. A system from Thermo Fisher was used consisting of a Vanquish Flex pump and a Orbitrap Exploris 120 mass spectrometer.

Stepwise synthesis of the Boc-protected trisbenzimidazole alcohol 16

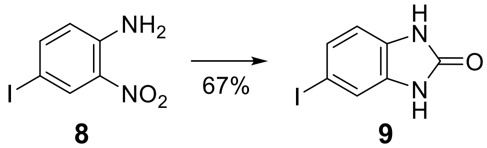
4-Iodo-2-nitroaniline 8 [4]



7 **8**

A mixture of 2-nitroaniline **7** (5.00 g, 36.20 mmol, 1.00 eq.) and sodium acetate (3.12 g, 38.03 mmol, 1.05 eq.) dissolved in acetic acid (50 mL) was treated dropwise over a period of 20 min with a solution of ICl (1.99 mL, 38.05 mmol, 1.05 eq.) in acetic acid (5.37 mL). The solution was then heated to 90 °C for 30 min. After cooling, the slurry was poured into ice-water to afford a red-brown precipitate that was filtered and dried in vacuum to obtain the desired product (9.00 g, 94%). R_f = 0.16 (cyclohexane/EtOAc 8:1). Mp. 121 - 123 °C. ^1H-NMR (400 MHz, DMSO-*d*$_6$): 8.21 (d, *J* = 2.1 Hz, 1 H), 7.61 (dd, *J* = 8.9, 2.1 Hz, 1 H), 7.55 (s, 2 H, NH_2), 6.87 (d, *J* = 8.9 Hz, 1 H) ppm. ^{13}C-NMR (126 MHz, DMSO-*d*$_6$): 145.6, 143.2, 132.9, 131.4, 121.5, 74.8 ppm. MS (ESI-): *m/z* calcd. for $C_6H_4IN_2O_2$ [M-H$^+$]: 262.93; found 263.12. HRMS (MALDI): *m/z* calcd. for $C_6H_6IN_2O_2$ [M+H$^+$]: 264.94740; found 264.94689. The NMR spectra are very similar to data from ref. 4 measured in $CDCl_3$.

5-Iodo-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one 9 [4]

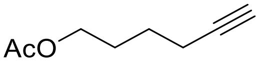


8 **9**

A solution of $SnCl_2$ $2H_2O$ (25.42 g, 11.26 mmol, 3.50 eq.) in 12 N HCl (47.60 mL) was added dropwise over 45 min into a solution of compound **8** (8.50 g, 32.19 mmol, 1.00 eq.) in 17 mL of 12 N HCl and stirred for 2 h at 65 °C and for 1 h at 25 °C. The mixture was cooled to 5 °C for 16 h. The precipitate was filtered off, dissolved in cold H_2O (90 mL) and treated with a solution of NaOH (17 g) in 35 mL H_2O. Once again, the resulting precipitate was filtered off, washed with cold H_2O (2 x 20 mL) and dried in vacuum to provide 4-iodobenzene-1,2-diamine (5.90 g, 78%). Since the compound is unstable at 25 °C, it was used directly in the subsequent reaction without further purification: The diamine was dissolved in THF (47 mL), treated with 1,1-carbonyldiimidazole (6.16 g, 37.98 mmol, 1.5 eq.), stirred at 25 °C for 18 h and evaporated. The residue was dissolved in Et_2O (200 mL) and then it was extracted with 1 N NaOH (3 x 80 mL). The combined aqueous phases were acidified with 12 N HCl. The precipitate was filtered off, washed with cold H_2O (4 x 20 mL) and dried in vacuum to afford product **9** as a colorless solid (5.60 g, 67%). R_f = 0.22 (cyclohexane/EtOAc 2:1). Mp. 338 - 339 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): 10.75 (s, 1 H, NH), 10.72 (s, 1 H, NH), 7.25 (dd, J = 8.1, 1.7 Hz, 1 H), 7.21 (d, J = 1.8 Hz, 1 H), 6.78 (d, J = 8.1 Hz, 1 H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): 154.8, 131.5, 129.45, 128.8, 116.5, 110.7, 82.7 ppm. MS (ESI-): *m/z* calcd. for $C_7H_4IN_2O$ [M-H$^+$]: 258.94; found 258.92 [M-H$^+$]. HRMS (MALDI): *m/z* calcd. for $C_7H_6IN_2O$ [M+H$^+$]: 260.95248; found 260.95207. The NMR spectra are consistent with data from ref. 4.

4-Iodobenzene-1,2-diamine: ^{1}H-NMR (400 MHz, DMSO-d_6): 6.82 (d, J = 2.1 Hz, 1 H), 6.66 (dd, J = 8.1, 2.0 Hz, 1 H), 6.34 (d, J = 8.1 Hz, 1 H), 4.75 (s, 4 H, NH_2) ppm. The NMR spectrum is consistent with data from ref. 4.

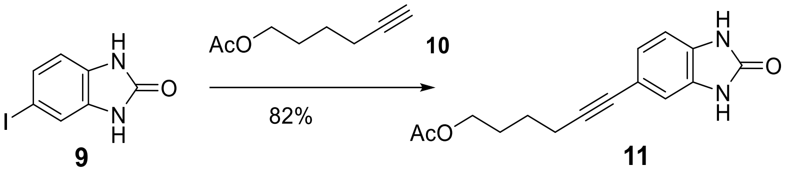
Hex-5-yn-1-yl acetate 10 [14]



10

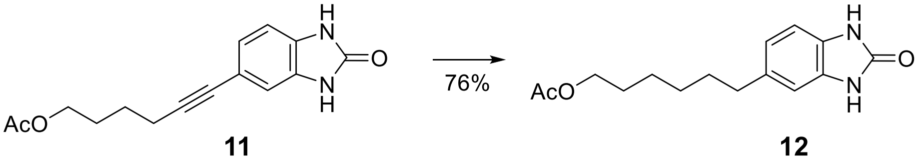
A solution of 5-hexyn-1-ol (1.50 g, 15.3 mmol, 1.00 eq.), acetic anhydride (1.65 mL, 17.5 mmol, 1.12 eq.) and pyridine (1,40 mL, 17.5 mmol, 1.12 eq.) was stirred at room temperature for 3 h. The reaction was monitored by TLC. After completion, the mixture was poured into 1M HCl (20 mL) and extracted with Et_2O (3 x 20 mL). The combined organic phases were washed with aq. NH_4Cl (3 x 20 mL), aq. $NaHCO_3$ (20 mL), aq. NaCl (20 mL) and they were dried over $MgSO_4$. After filtration the solution was concentrated under vacuum. A subsequent vacuum distillation was performed in order to obtain ester **10** as a colorless and solvent-free liquid (2.10 g, 96%). R_f = 0.91 (cyclohexane/EtOAc 1:1). Bp. 82 °C (24 mbar). ^{1}H-NMR (400 MHz, $CDCl_3$): 4.02 (t, J = 6.5 Hz, 2 H), 2.17 (td, J = 7.0, 2.6 Hz, 2 H), 1.98 (s, 3H), 1.90 (t, J = 2.7 Hz, 1 H), 1.77 – 1.64 (m, 2 H), 1.60 – 1.45 (m, 2 H) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$): 171.1, 83.8, 68.7, 63.9, 27.6, 24.9, 20.9, 18.0 ppm. IR (ṽ, cm^{-1})**:** 3290 (w), 2945 (w), 1735 (s), 1366 (m), 1234 (s), 1043 (m), 634 (m). The ^{1}H NMR spectrum is consistent with data from ref. 14.

6-(2-Oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)hex-5-yn-1-yl acetate 11



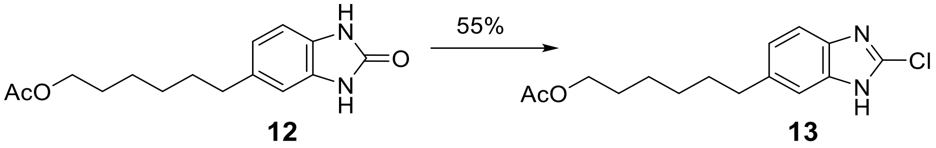
Compound **9** (4.00 g, 15.4 mmol, 1.00 eq.) and alkyne **10** (4.09 g, 29.2 mmol, 1.9 eq.) were dissolved in DMF (50 mL). Et_3N (15.7 mL, 111 mmol, 7.2 eq.), CuI (0.70 g, 3.68 mmol, 0.24 eq.) and $Pd(Ph_3P)_4$ (2.13 g, 1.84 mmol, 0.12 eq.) were added under argon. The solution was degassed and flushed with argon multiple times. The mixture was stirred for 20 h at 80 °C. The progress of the reaction was monitored by TLC and after completion the mixture was diluted with aqueous $NaHCO_3$ solution (50 mL) and extracted with EtOAc (3 x 40 mL). The organic phases were combined, washed with H_2O (3 x 40 mL) and dried with $NaSO_4$. The solvent was removed in vacuum. The purification of the product was performed by column chromatography (DCM/MeOH 20:1) and a yellowish solid of **11** was obtained (3.41 g, 82%). R_f = 0.25 (DCM/MeOH 20:1). Mp. 191 - 193 °C. ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 10.73 (s, 1H, NH), 10.65 (s, 1H, NH), 6.97 (dd, *J* = 7.9, 1.6, Hz, 1 H), 6.89-6.85 (m, 2 H), 4.04 (t, *J* = 6.5 Hz, 2 H), 2.41 (t, *J* = 7.0 Hz, 2 H), 2.00 (s, 3 H), 1.77- 1.65 (m, 2 H), 1.62-1.51 (m, 2 H) ppm. ^{13}C-NMR (126 MHz, DMSO-*d*$_6$): 170.9, 155.7, 130.2, 130.0, 124.7, 115.4, 111.4, 109.0, 88.2, 81.9, 63.9, 27.9, 25.3, 21.2, 18.8 ppm. MS (ESI-): *m/z* calcd. for $C_{15}H_{15}N_2O_3$ [M-H$^+$]: 271.11; found 271.07. HRMS (MALDI): *m/z* calcd. for $C_{15}H_{17}N_2O_3$ [M+H$^+$]: 273.12392; found 273.12395.

6-(2-Oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)hexyl acetate 12



Compound **11** (3.00 g, 11.0 mmol, 1.00 eq.) was dissolved in 60 mL dry MeOH. 10% Pd/C (0.30 g) was added and the solution was stirred for 10 min under argon. After that, Et_3SiH (17.6 mL, 110 mmol, 10.00 eq.) was added dropwise, and the reaction was stirred at room temperature for 2 h. The solution was filtrated through celite and the solvent was evaporated in vacuum to give a colorless solid product. Further purification by column chromatography (DCM / MeOH 20: 1) was needed to remove the excess of Et_3SiH. Yield of compound **12**: 2.30 g (76%). R_f = 0.25 (DCM/MeOH 20:1). Mp. 177 °C. ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 10.48 (s, 1 H, NH), 10.46 (s, 1 H, NH), 6.83 - 6.81 (m, 1 H), 6.75 – 6.72 (m, 2 H) 3.97 (t, *J* = 6.7 Hz, 2 H), 2.54 (t, *J* = 7.2 Hz, 2 H, overlap with DMSO), 1.99 (s, 3 H), 1.59 – 1.47 (m, 4 H), 1.38 – 1.22 (m, 4 H) ppm. ^{13}C-NMR (400 MHz, DMSO-*d*$_6$): 170.3, 155.5, 134.5, 129.8, 127.6, 120.3, 108.3, 108.11, 63.8, 35.1, 31.4, 28.2, 28.0, 25.2, 20.6 ppm. MS (ESI-): *m/z* calcd. for $C_{15}H_{19}N_2O_3$ [M-H$^+$]: 275.14; found 275.07. HRMS (MALDI): *m/z* calcd. for $C_{15}H_{21}N_2O_3$ [M+H$^+$]: 277.15522; found 277.15475.

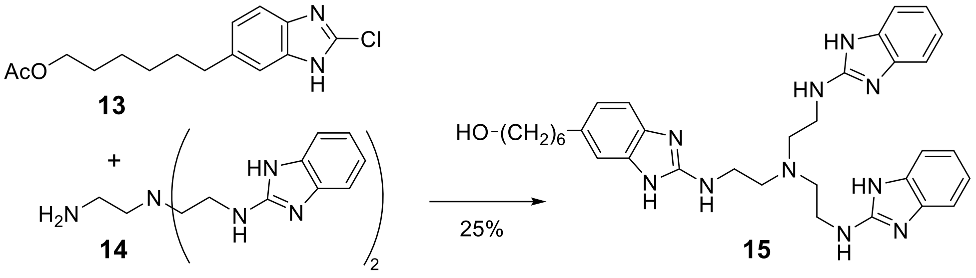
6-(2-Chloro-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)hexyl acetate 13



The chlorination of the compound **12** is a microwave assisted reaction. The material (2.00 g, 7.24 mmol, 1.00 eq.) was dissolved in CH_3CN (50 mL) and $POCl_3$ (2.64 ml, 28.9 mmol, 4.00 eq.) was added dropwise. The vial was flushed properly with argon, sealed and then placed in a microwave reactor. The mixture was stirred for 15 minutes at 140 °C and the progress of the reaction was checked by TLC. After completion, the solution was poured into ice-water and neutralized by aq. $NaHCO_3$. The aqueous solution was extracted by EtOAc (3 x 20 mL). The combined organic phases were dried with Na_2SO_4, filtrated and evaporated in vacuo to give a colorless oil. The product was cooled to 5 °C for 20 h and colorless crystals of **13** appeared (1.17 g, 55%). R_f = 0.13 (cyclohexane/EtOAc 2:1). Mp. 161 °C – 162 °C. ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 13.07 (broad, 1 H, NH), 7.41 (d, *J* = 8.2 Hz, 1 H), 7.30 (bs, 1 H), 7.07 (dd, *J* = 8.2, 1.6 Hz, 1 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 2.67 (t, *J* = 7.6 Hz, 2 H), 2.00 (s, 3 H), 1.71-1.50 (m, 4 H), 1.43-1.25 (m, 4 H) ppm, mixture of 2 tautomers. MS (ESI+): *m/z* calcd. for

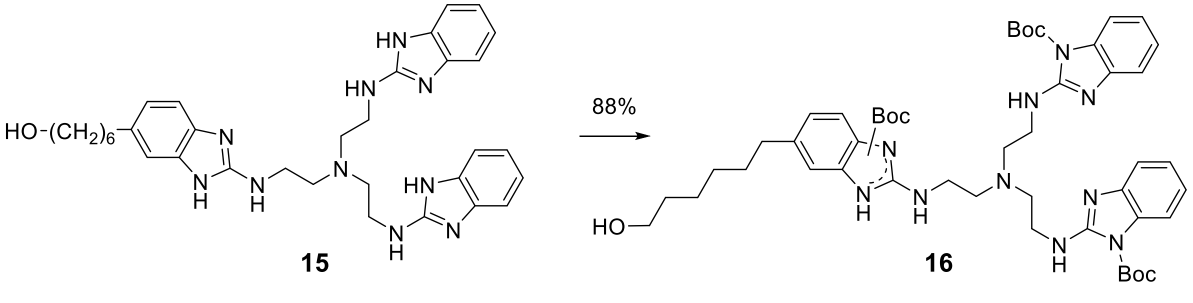
$C_{15}H_{20}ClN_2O_2$ [M+H$^+$]: 295.12; found 295.05. HRMS (MALDI): *m/z* calcd. for $C_{15}H_{20}ClN_2O_2$ [M+H$^+$]: 295.12133; found 295.12088.

Trisbenzimidazole alcohol 15



This is a microwave assisted reaction where compound **13** (0.40 g, 1.35 mmol, 1 eq.) and compound **14** (0.76 g, 2.08 mmol, 1.54 eq.) were dissolved in 12.5 mL ethylene glycol. The solution was heated to 190 °C for 15 min in a closed vessel and the progress was monitored by TLC. After completion, the mixture was neutralized by aqueous $NaHCO_3$ and it was extracted by EtOAc multiple times. The combined organic phases were washed with brine and dried over $MgSO_4$. According to TLC and mass spectrometry, the crude material (0.30 g) consisted of the acetyl protected and the unprotected product in a 1:1 ratio. To complete the removal of acetyl, the mixture was dissolved in a solution of LiOH (45 mg, 1.88 mmol) in 5 mL THF:H_2O (4:1). The solution was transferred into a vial and was placed in a microwave reactor. The reaction was heated in the closed vessel to 110° C for 15 min. The completion of the reaction was monitored by TLC and then the solution was extracted by EtOAc (2 x 10 mL). The combined organic phases were dried over $MgSO_4$ and the filtrate was evaporated in vacuo. Subsequent purification by column chromatography (DCM/MeOH 13:1+2% NH_3) yielded compound **15** as a brownish foam (0.20 g, 25%). *Rf* = 0.12 (DCM/MeOH 10:1+2% NH_3). ^{1}H-NMR (500 MHz, DMSO-*d*$_6$): 7.17-7.12 (m, 4H), 7.03 (d, *J* = 7.9 Hz, 1 H), 6.96 (d, *J* = 1.6 Hz, 1 H), 6.90-6.85 (m, 4 H), 6.70 (dd, *J* = 8.0, 1.6 Hz, 1 H), 6.59 (s, 2 H, NH), 6.53 (s, 1 H, NH), 3.45-3.38 (m, 8 H), 2.79 (t, *J* = 6.6 Hz, 6 H), 2.55 (t, *J* = 7.6 Hz, 2 H), 1.58 – 1.52 (m, 2 H), 1.44 – 1.39 (m, 2 H), 1.34-1.23 (m, 4 H) ppm. ^{13}C-NMR (126 MHz, DMSO-*d*$_6$): 156.18, 156.10, 139.1, 137.1, 133.8, 120.0, 119.6, 112.0, 61.2, 54.21, 54.18, 49.1, 41.12, 41.16, 35.9, 33.0, 32.4, 29.1, 25.9 ppm. MS (ESI-): *m/z* calcd. for $C_{33}H_{41}N_{10}O$ [M-H$^+$]: 593.35; found 593.27. HRMS (MALDI): *m/z* calcd. for $C_{33}H_{43}N_{10}O$ [M+H$^+$]: 595.36213; found 595.36169.

Boc-protected trisbenzimidazole alcohol 16

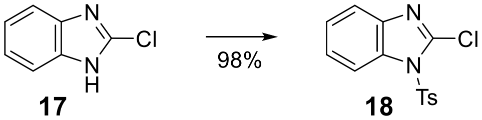


Compound **15** (0.20 g, 0.33 mmol, 1.00 eq.) was dissolved in 5 mL DMF. The solution was degassed and flushed with argon. DIPEA (0.2 mL, 1.17 mmol, 3.5 eq.) was added and after stirring for 1 min under argon, Boc_2O (0.25 mL, 1.17 mmol, 3.5 eq.) was added dropwise. The solution was stirred for 3h. The completion of the reaction was monitored by TLC. The solution was diluted with EtOAc, washed with aqueous $NaHCO_3$ and reextracted with EtOAc. The combined organic phases were washed with brine (2 x 10 mL) and dried by $MgSO_4$. The filtrated solution was evaporated in vacuo. The crude material was purified by column chromatography (DCM/MeOH

25:1 to 9:1) to yield product **16** as a colorless foam (0.26 g, 88%). *Rf* = 0.34 (DCM/MeOH 20:1 + 2% NH_3). ^{1}H-NMR (500 MHz, DMSO-d_6, mixture of two isomers): 7.55 (2t, *J* = 5 Hz, together 2 H, NH), 7.53 – 7.49 (m, 2.5 H), 7.47 (t, *J* = 5.4 Hz, 0.5 H, NH), 7.39-7.34 (m, 1 H), 7.20 (dd, *J* = 7.8, 1.7 Hz, 2 H), 7.12-7.08 (m, 2.5 H), 6.99-6.95 (m, 2.5 H), 6.92 (dd, *J* = 8.0, 1.7 Hz, 0.5 H), 6.77 (dd, *J* = 8.2, 1.7 Hz, 0.5 H), 4.35 (t, *J* = 5.1 Hz, 1 H, OH), 3.59 – 3.51 (m, 6 H), 3.37 (q, 2 H, hidden by H_2O), 2.90- 2.83 (m, 6 H), 2.58 and 2.54 (2t, *J* = 7.4 Hz, together 2 H), 1.58-1.53 (m, 2 H), 1.47 (s, 9 H), 1.46 (s, 18 H), 1.42-1.37 (m, 2 H), 1.33-1.21 (m, 4 H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6, mixture of two isomers): 154.21, 154.12, 153.9, 150.6, 143.34, 143.24, 141.3, 138.6, 134.3, 130.91, 130.82, 128.9, 124.54, 124.34, 120.5, 120.27, 116.1, 115.94, 115.68, 114.11, 114.0, 113.7, 85.73, 85.49, 61.19, 61.16, 54.08, 54.02, 41.53, 35.73, 35.57, 33.02, 32.98, 31.91, 31.81, 31.75, 28.91, 28.85, 27.81, 27.77, 25.87, 25.84 ppm. MS (ESI+): *m/z* calcd. for $C_{48}H_{67}N_{10}O_7$ [M+H$^+$]: 895.52; found 895.43. HRMS (MALDI): *m/z* calcd. for $C_{48}H_{66}KN_{10}O_7$ [M+K$^+$]: 933.47530; found 933.47413.

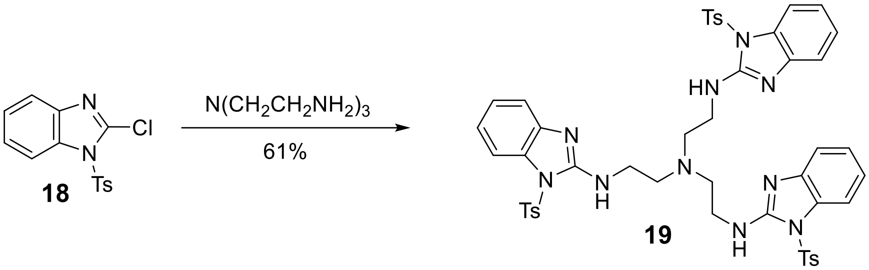
Synthesis of phosphoramidites 2 – 5 via statistical iodination

2-Chloro-1-tosyl-1*H*-benzo[*d*]imidazole 18 [6]

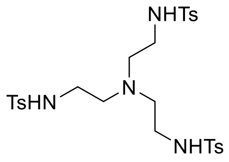


To a suspension of 2-chlorobenzimidazole **17** (10.0 g, 65.5 mmol, 1 eq.) in 100 mL DCM was added at 0 °C triethylamine (8.06 g, 11.1 mL, 79.6 mmol, 1.2 eq.) and tosyl chloride (12.7 g, 66.6 mmol, 1.02 eq.). The mixture was stirred for 2 h at room temperature. Then it was washed with water (2x) and with brine and dried with $MgSO_4$. The solvent was evaporated. The remaining colorless oil crystallized upon standing. It was washed with pentane to yield compound **18** in form of colorless crystals (19.68 g, 98%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.11 (ddd, *J* = 8.4, 1.3, 0.8 Hz, 1H), 7.92 – 7.88 (m, 2H), 7.63 (ddd, *J* = 7.8, 1.5, 0.7 Hz, 1H), 7.44 – 7.33 (m, 2H), 7.33 – 7.28 (m, 2H), 2.40 (s, 3H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 146.77, 141.03, 138.48, 134.73, 133.90, 130.39, 127.67, 125.75, 125.26, 119.99, 114.01, 21.85 ppm. MS (ESI+): *m/z* calcd. for $C_{13}H_{12}ClN_2O_2S$ [M+H$^+$]: 307.03; found 306.95. The NMR spectra are consistent with data from ref. 6.

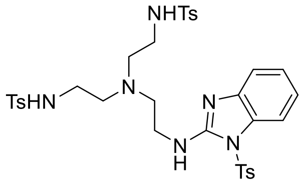
Tosylated trisbenzimidazole 19



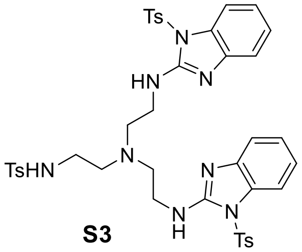
To a solution of benzimidazole **18** (7.06 g, 23.0 mmol, 3.3 eq.) in 60 mL of benzene was added Cs_2CO_3 (8.25 g, 25.3 mmol, 3.66 eq.) and TREN (1.01 g, 6.90 mmol, 1 eq.). The resulting suspension was heated to reflux for 4 h. After evaporation of the solvent, water and DCM were added. The phases were separated, the aqueous phase extracted with DCM (3x) and dried with $MgSO_4$. After evaporation of DCM, the crude product was submitted to column chromatography (EtOAc/cyclohexane 2:1 → EtOAc/MeOH 10:1). Compounds **S1** (together with detosylated benzimidazole **17**), **S2**, and **S3** were eluted in this order. Trisbenzimidazole **19** could be isolated from the last fractions as a colorless foam (4.00 g, 61%). **19**: ^{1}H-NMR (400 MHz, $CDCl_3$): 7.80 (d, *J* = 8.3 Hz, 6H), 7.68 (d, *J* = 7.6 Hz, 3H), 7.29 (d, *J* = 7.5 Hz, 3H), 7.18 – 7.12 (m, 9H), 7.03 (td, *J* = 8.0, 1.2 Hz, 3H), 6.87 (t, *J* = 5.4 Hz, 3H), 3.71 (q, *J* = 6.1 Hz, 6H), 3.02 (t, *J* = 6.2 Hz, 6H), 2.25 (s, 9H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.25, 145.97, 142.77, 134.56, 131.07, 130.21, 127.00, 124.83, 121.29, 116.90, 112.53, 54.18, 41.82, 21.67 ppm. MS (ESI+): *m/z* calcd. for $C_{48}H_{49}N_{10}O_6S_3$ [M+H$^+$]: 957.30; found 957.48. HRMS (MALDI): *m/z* calcd. for $C_{48}H_{50}N_{10}O_6S_3$ [M+2H$^+$]: 479.15385; found 479.1533.



S1



S2



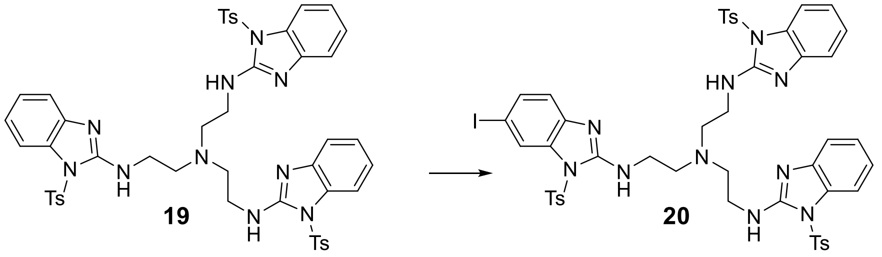
S3

S1: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.68 – 7.64 (m, 6H), 7.36 – 7.33 (m, 9H), 2.66 (q, *J* = 6.2 Hz, 6H), 2.37 (s, 9H), 2.22 (t, *J* = 6.3 Hz, 6H) ppm, together with signals of 2-chlorobenzimidazole **17** at 13.23 (br. s), 7.51 (m) and 7.22 (m) ppm.

S2: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.82 (d, *J* = 8.3 Hz, 2H), 7.68 – 7.60 (m, 5H), 7.45 (t, *J* = 5.8 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 8.5 Hz, 4H), 7.18 (m, 1H), 7.14 (ddd, *J* = 7.9, 6.4, 1.2 Hz, 1H), 7.04 (ddd, *J* = 7.9, 7.2, 1.5 Hz, 1H), 6.98 (t, *J* = 5.5 Hz, 1H), 3.31 (m, overlap with water, 2H), 2.78 (q, *J* = 6.7 Hz, 4H), 2.59 (t, *J* = 6.5 Hz, 2H), 2.47 (t, *J* = 6.5 Hz, overlap with DMSO, 4H), 2.32 (s, 3H), 2.29 (s, 6H) ppm. Sample contains EtOAc and other impurities.

S3: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.83 (d, *J* = 8.4 Hz, 4H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.51 (t, *J* = 5.8 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 4H), 7.21 – 7.16 (m, 4H), 7.12 (ddd, *J* = 7.8, 7.6, 1.2 Hz, 2H), 7.07 (t, *J* = 5.4 Hz, 2H), 7.03 (ddd, *J* = 7.9, 7.3, 1.4 Hz, 2H), 3.47 (q, *J* = 6.3 Hz, 4H), 2.95 (q, *J* = 6.9 Hz, 2H), 2.77 (t, *J* = 6.7 Hz, 4H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.23 (s, 3H), 2.22 (s, 6H) ppm.

Iodo-trisbenzimidazole 20



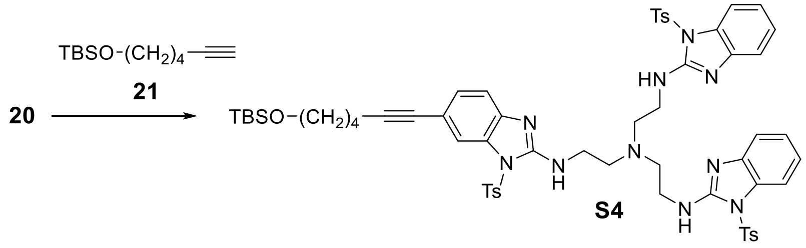
Trisbenzimidazole **19** (3.56 g, 3.72 mmol, 1 eq.) was dissolved in 50 mL HOAc. After addition of *N*-iodosuccinimide (0.84 g, 3.72 mmol, 1 eq.) the resulting solution was stirred at room temperature overnight. It was then diluted with EtOAc. Crushed ice and 50 mL of 30% aqueous NaOH were added and the phases were separated. The aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with conc. aqueous $NaHCO_3$ until gas evolution had ceased, washed with brine and dried with $MgSO_4$. After evaporation of the solvent, a brownish foam of crude **11** (3.80 g) was obtained and used in the next step without purification. MS (ESI+): *m/z* calcd. for $C_{48}H_{48}IN_{10}O_6S_3$ [M+H$^+$]: 1083.20; found 1083.37. HRMS (ESI-TOF): *m/z* calcd. for $C_{48}H_{49}IN_{10}O_6S_3$: 542.1016 [M+2H$^+$]; found 542.1011.

Silylation of hex-5-yn-1-ol yielding compound 21 [15]

$TBSO\text{-}(CH_2)_4\text{—}\equiv$ **21**

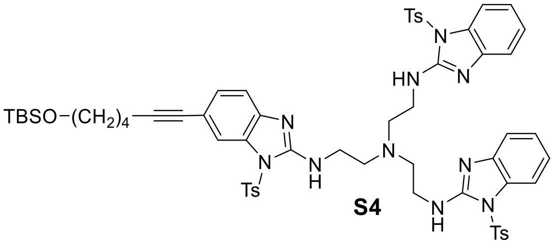
Hex-5-yn-1-ol (5.00 g, 5.62 mL, 50.9 mmol, 1 eq.) was dissolved in DMF (50 mL), cooled to 0°C and, after addition of imidazole (3.81 g, 56 mmol, 1.1 eq.) and TBS-Cl (8.41 g, 56 mmol, 1.1 eq.), stirred for 1 h at room temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with diethyl ether. The combined ether phases were washed with water and with brine and dried with $MgSO_4$. Evaporation of the solvent yielded a colorless oil of **21** (10.82 g, quant.) which was used in the next step without purification. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.63 (t, *J* = 6.0 Hz, 2H), 2.21 (td, *J* = 6.8, 2.6 Hz, 2H), 1.94 (t, *J* = 2.6 Hz, 1H), 1.68 – 1.54 (m, 4H), 0.89 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 84.69, 68.39, 62.74, 31.96, 26.10, 25.11, 18.49, 18.37, -5.16. ^{29}Si-NMR (80 MHz, $CDCl_3$): 18.64 ppm. The ^{1}H and ^{13}C NMR spectra are consistent with data from ref. 15.

Sonogashira coupling of iodo-trisbenzimidazole 20 and alkyne 21



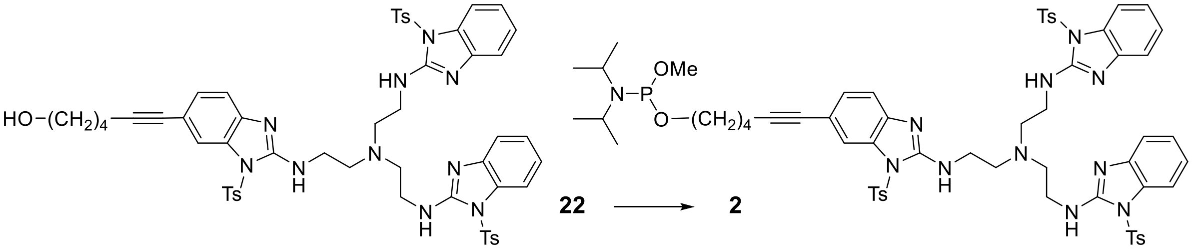
Crude compound **20** (1.46 g (from 1.36 g **19**, 1.43 mmol), 1 eq.), $Pd(PPh_3)_2Cl_2$ (47 mg, 0.067 mmol, 4.7 mol %) and CuI (26 mg, 0.137 mmol, 9.6 mol %) were placed in a round bottom flask, evacuated and filled with argon. Alkyne **21** (0.57 g, 2.7 mmol, 1.89 eq.) was dissolved in a second flask in 20 mL DMF and 2 mL Et_3N. It was degassed by slowly bubbling argon through the solution for 15 min and then transferred into the flask with compound **20**. The reaction mixture was stirred at room temperature overnight. Water was added and the mixture was extracted twice with EtOAc. The combined organic phases were washed with water and with brine. After drying with $MgSO_4$ the solvent was evaporated and the residue was partially purified by chromatography (SiO_2, EtOAc/ cyclohexane 2:1), mainly to remove products of oxidative alkyne dimerization. Some pure fractions of **S4** were used for characterization. Most of the material, however, was a mixture of **S4** with analogs containing none (**19**) or two silylated side chains. After removal of the solvent, crude product **S4** was obtained as a colorless foam and directly used in the next step. The final purification was possible at this stage (see below). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.79 (d, *J* = 8.5 Hz, 6H), 7.73 (d, *J* = 0.9 Hz, 1H), 7.68 (dd, *J* = 8.1, 0.6 Hz, 2H), 7.28 (dd, *J* = 7.9, 0.6 Hz, 2H), 7.23 – 7.12 (m, 10H), 7.04 (td, J = 7.7, 1.2 Hz, 2H), 6.90 – 6.83 (m, 3H), 3.74 – 3.65 (m, 8H), 3.04 – 2.97 (m, 6H), 2.47 – 2.42 (m, 2H), 2.28 (s, 3H), 2.27 (s, 6H), 1.75 – 1.65 (m, 4H), 0.91 (s, 9H), 0.07 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.84, 152.25, 146.13, 146.01, 142.77, 142.45, 134.59, 134.44, 131.09, 130.94, 130.33, 130.24, 128.92, 127.05, 127.03, 124.87, 121.33, 116.94, 116.78, 116.58, 115.61, 112.55, 88.90, 81.35, 62.91, 54.24, 54.19, 41.84, 32.24, 26.14, 25.47, 21.71, 19.46, 18.51, -5.10 ppm. MS (ESI+): *m/z* calcd. for $C_{60}H_{71}N_{10}O_7S_3Si$ [M+H$^+$]: 1167.44; found 1167.55. HRMS (ESI-TOF): *m/z* calcd. for $C_{60}H_{72}N_{10}O_7S_3Si$ [M+2H$^+$]: 584.2253; found 584.2256.

Desilylation of intermediate S4 forming compound 22



The crude TBS protected compound **S4** (see above) was dissolved in 5 mL THF. After addition of 15 mL HOAc and 5 mL water, the solution was stirred at room temperature overnight. Then it was diluted with EtOAc. Crushed ice and 15 mL of 30% aqueous NaOH were added and the phases were separated. The aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with conc. aqueous $NaHCO_3$ until gas evolution had ceased, washed with brine and dried with $MgSO_4$. After removal of the solvent, the residue was purified by chromatography (SiO_2, DCM/MeOH 30:1). By evaporation of the pure fractions, product **22** was obtained as a colorless foam (340 mg, 23% over three steps from trisbenzimidazole **19**). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.84 (d, *J* = 8.4 Hz, 6H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.57 (d, *J* = 1.4 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 4H), 7.18 – 7.07 (m, 9H), 7.02 (td, *J* = 7.6, 1.5 Hz, 2H), 4.43 (t, *J* = 5.2 Hz, 1H), 3.62 (q, *J* = 6.3 Hz, 6H), 3.50 – 3.42 (m, 2H), 2.93 (t, *J* = 6.8 Hz, 6H), 2.46 – 2.40 (m, 2H), 2.17 (s, 3H), 2.16 (s, 6H), 1.69 – 1.55 (m, 4H) ppm. ^{13}C-NMR (101 MHz, DMSO-*d*$_6$): 152.70, 151.96, 146.43, 146.21, 142.74, 142.60, 133.46, 133.29, 130.43, 130.40, 130.38, 130.27, 128.44, 126.62, 124.74, 120.77, 116.22, 115.35, 114.60, 112.21, 89.17, 81.00, 60.24, 52.30, 31.78, 25.00, 20.95, 20.92, 18.58, 14.07 ppm. MS (ESI+): *m/z* calcd. for $C_{54}H_{57}N_{10}O_7S_3$ [M+H$^+$]: 1053.36; found 1053.51. HRMS (ESI-TOF): *m/z* calcd. for $C_{54}H_{57}N_{10}O_7S_3$ [M+H$^+$]: 1053.3568; found 1053.3565.

Phosphoramidite 2



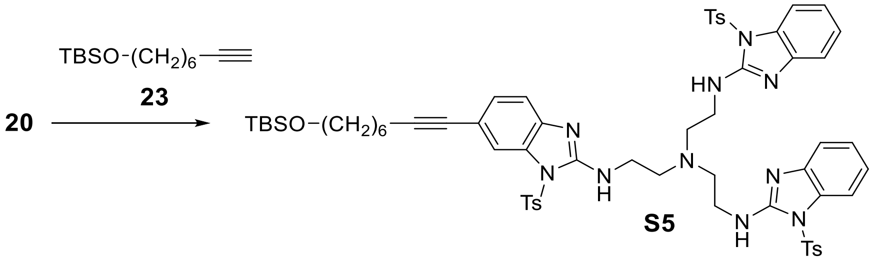
A solution of compound **22** (0.35 g, 0.33 mmol, 1.0 eq.) in 10 mL dry DCM and DIPEA (0.22 mL, 1.3 mmol, 4 eq.) was cooled to 0 °C. Methyl *N*,*N*-diisopropylchlorophosphoramidite (0.13 mL, 0.67 mmol, 2.0 eq.) was slowly added drop by drop. The reaction mixture was stirred at 0 °C for 20 min and at room temperature for 30 min. It was then quenched by addition of saturated aqueous $NaHCO_3$ solution and extracted with DCM. The organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure (water bath at 30 °C). The residue war purified by chromatography (SiO_2, EtOAc/cyclohexane 2:1 + 2% Et_3N). Lyophilization of the product fractions from benzene yielded 350 mg (87%) of phosphoramidite **2** as a colorless amorphous powder. ^{1}H-NMR (400 MHz, C_6D_6): 8.36 (d, *J* = 1.5 Hz, 1H), 8.00 (dd, *J* = 7.9, 1.3 Hz, 2H), 7.81 – 7.76 (m, 6H), 7.46 (dd, *J* = 7.7, 1.3 Hz, 2H), 7.38 (dd, J = 8.2, 1.6 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.02 (td, *J* = 7.6, 1.3 Hz, 2H), 6.96 (td, *J* = 7.7, 1.3 Hz, 2H), 6.93 – 6.85 (m, 1H), 6.88 (t, *J* = 5.6 Hz, 2H), 6.55 (d, *J* = 8.3 Hz, 4H), 6.50 (d, *J* = 8.2 Hz, 2H), 3.77 – 3.68 (m, 1H), 3.67 – 3.59 (m, 2H), 3.58 – 3.48 (m, 6H), 3.35 (d, *J* = 12.8 Hz, 3H), 2.58 – 2.50 (m, 6H), 2.32 (t, *J* = 6.8 Hz, 2H), 1.79 – 1.63 (m, 4H), 1.62 (s, 6H), 1.60 (s, 3H), 1.20 (d, *J* = 6.8 Hz, 6H), 1.18 (d, *J* = 6.8 Hz, 6H). ^{31}P-NMR (162 MHz, C_6D_6): 148.20 ppm.

Silylation of oct-7-yn-1-ol yielding compound 23 [16]

$\mathrm{TBSO{-}(CH_2)_6{-}{\equiv}}$ **23**

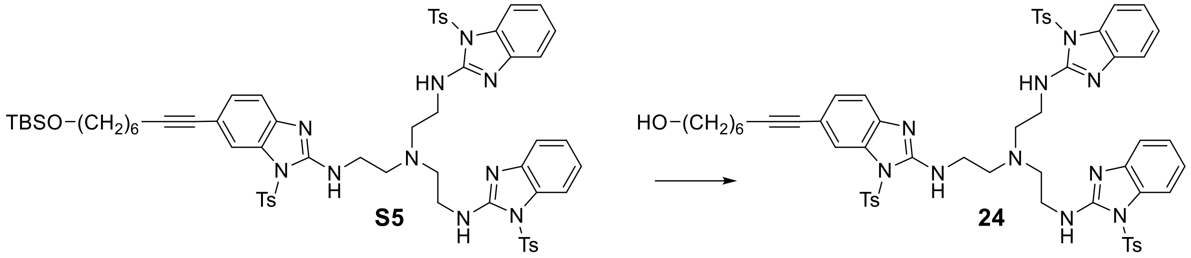
Oct-7-yn-1-ol (5.00 g, 39.6 mmol, 1 eq.) was dissolved in DMF (50 mL), cooled to 0°C and, after addition of imidazole (2.97 g, 43.6 mmol, 1.1 eq.) and TBS-Cl (6.54 g, 43.6 mmol, 1.1 eq.), stirred for 1 h at room temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with diethyl ether. The combined ether phases were washed with water and with brine and dried with $MgSO_4$. Evaporation of the solvent yielded a colorless oil of **23** (9.53 g, quant.) which was used in the next step without purification. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.60 (t, *J* = 6.5 Hz, 2H), 2.18 (td, *J* = 7.1, 2.6 Hz, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.57 – 1.48 (m, 4H), 1.46 – 1.31 (m, 4H), 0.89 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 84.86, 68.24, 63.30, 32.85, 28.69, 28.63, 26.13, 25.47, 18.52, 18.51, -5.12 ppm. ^{29}Si-NMR (80 MHz, $CDCl_3$): 18.41 ppm. The ^{1}H and ^{13}C NMR spectra are consistent with data from ref. 16.

Sonogashira coupling of iodo-trisbenzimidazole 20 and alkyne 23



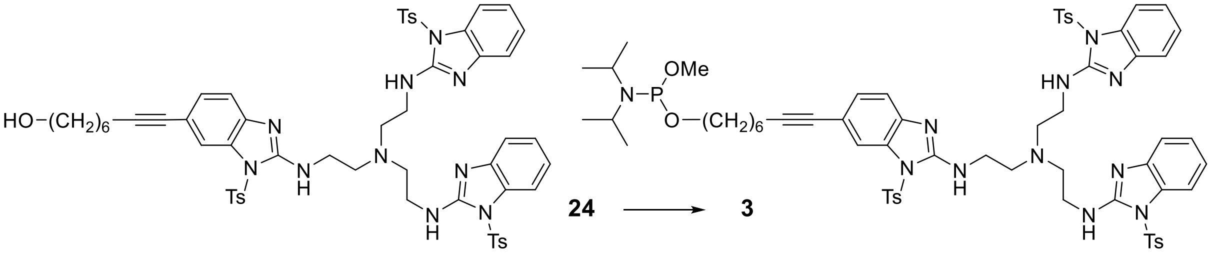
Crude compound **20** (2.34 g (from 2.19 g **19**, 2.29 mmol), 1 eq.), $Pd(PPh_3)_2Cl_2$ (76 mg, 0.11 mmol, 4.8 mol %) and CuI (41 mg, 0.21 mmol, 9.2 mol %) were placed in a round bottom flask, evacuated and filled with argon. Alkyne **23** (1.04 g, 4.32 mmol, 1.9 eq.) was dissolved in a second flask in 30 mL DMF and 3 mL Et_3N. It was degassed by slowly bubbling argon through the solution for 15 min and then transferred into the flask with compound **20**. The reaction mixture was stirred at room temperature overnight. Water was added and the mixture was extracted twice with EtOAc. The combined organic phases were washed with water and with brine. After drying with $MgSO_4$ the solvent was evaporated and the residue was partially purified by chromatography (SiO_2, EtOAc/ cyclohexane 2:1), mainly to remove products of oxidative alkyne dimerization. Some pure fractions of **S5** were used for characterization. Most of the material, however, was a mixture of **S5** with analogs containing none (**19**) or two silylated side chains. After removal of the solvent, crude product **S5** was obtained as a colorless foam and directly used in the next step. The final purification was possible at this stage (see below). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.81 – 7.77 (m, 6H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.67 (dd, *J* = 8.1, 0.6 Hz, 2H), 7.28 (dd, *J* = 7.9, 0.7 Hz, 2H), 7.22 – 7.11 (m, 10H), 7.03 (td, *J* = 7.9, 1.2 Hz, 2H), 6.90 – 6.83 (m, 3H), 3.73 – 3.66 (m, 6H), 3.63 (t, *J* = 6.5 Hz, 2H), 3.03 – 2.97 (m, 6H), 2.42 (t, *J* = 7.1 Hz, 2H), 2.27 (s, 3H), 2.27 (s, 6H), 1.63 (quintet, *J* = 7.9, 7.4 Hz, 2H), 1.58 – 1.52 (m, 2H), 1.52 – 1.44 (m, 2H), 1.44 – 1.36 (m, 2H), 0.90 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.83, 152.25, 146.13, 146.01, 142.77, 142.43, 134.59, 134.44, 131.09, 130.93, 130.32, 130.24, 128.92, 127.05, 127.02, 124.86, 121.33, 116.93, 116.81, 116.57, 115.61, 112.54, 89.08, 81.19, 63.36, 54.23, 41.84, 32.92, 29.05, 28.97, 26.14, 25.56, 21.73, 21.71, 19.61, 18.52, -5.10 ppm. MS (ESI+): *m/z* calcd. for $C_{62}H_{75}N_{10}O_7S_3Si$ [M+H$^+$]: 1195.47; found 1195.58. HRMS (ESI-TOF): *m/z* calcd. for $C_{62}H_{76}N_{10}O_7S_3Si$ [M+2H$^+$]: 598.2410; found 598.2400.

Desilylation of intermediate S5 forming compound 24



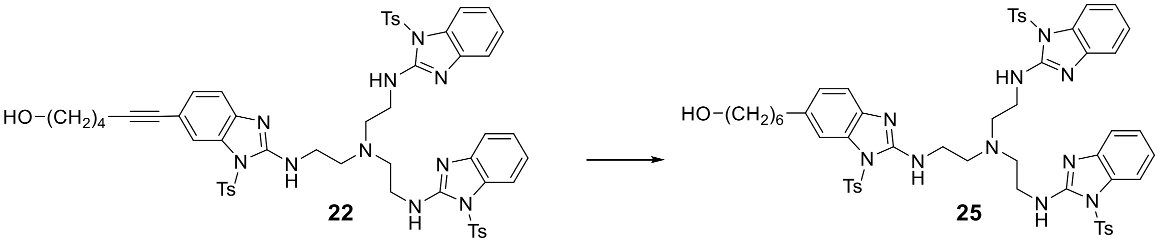
The crude TBS protected compound **S5** (see above) was dissolved in 5 mL THF. After addition of 15 mL HOAc and 5 mL water, the solution was stirred at room temperature overnight. Then it was diluted with EtOAc. Crushed ice and 15 mL of 30% aqueous NaOH were added and the phases were separated. The aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with conc. aqueous $NaHCO_3$ until gas evolution had ceased, washed with brine and dried with $MgSO_4$. After removal of the solvent, the residue was purified by chromatography (SiO_2, DCM/MeOH 30:1). By evaporation of the pure fractions, product **24** was obtained as a colorless foam (460 mg, 19% over three steps from trisbenzimidazole **19**). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.84 (d, *J* = 8.5 Hz, 6H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 1.5 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 4H), 7.18 – 7.07 (m, 9H), 7.02 (td, *J* = 7.6, 1.4 Hz, 2H), 4.35 (t, *J* = 5.2 Hz, 1H), 3.61 (q, *J* = 6.4 Hz, 6H), 3.40 (td, *J* = 6.4, 5.1 Hz, 2H), 2.97 – 2.88 (m, 6H), 2.41 (t, *J* = 7.0 Hz, 2H), 2.17 (s, 3H), 2.16 (s, 6H), 1.54 (quintet, *J* = 7.2 Hz, 2H), 1.48 – 1.38 (m, 4H), 1.38 – 1.29 (m, 2H) ppm. ^{13}C-NMR (101 MHz, DMSO-*d*$_6$): 152.70, 151.96, 146.43, 146.21, 142.74, 142.59, 133.46, 133.30, 130.43, 130.40, 130.38, 130.27, 128.43, 126.62, 124.74, 120.78, 116.23, 115.35, 114.60, 112.21, 89.14, 80.98, 60.67, 52.29, 52.24, 32.46, 28.33, 28.28, 25.04, 20.95, 20.92, 18.67 ppm. MS (ESI+): *m/z* calcd. for $C_{56}H_{61}N_{10}O_7S_3$ [M+H$^+$]: 1081.39; found 1081.54. HRMS (ESI-TOF): *m/z* calcd. for $C_{56}H_{62}N_{10}O_7S_3$ [M+2H$^+$]: 541.1977; found 541.1971.

Phosphoramidite 3



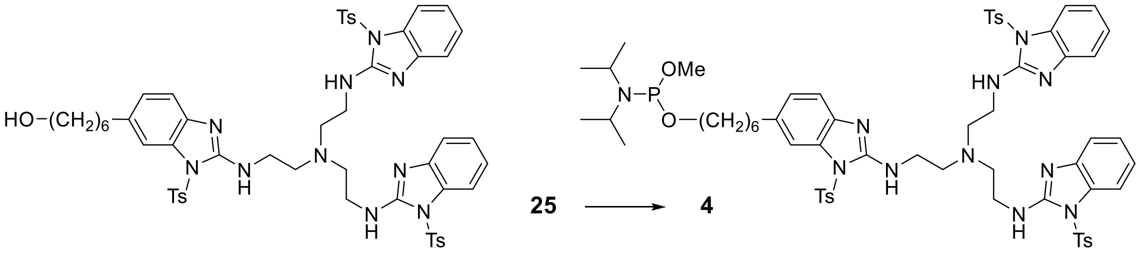
A solution of compound **24** (340 mg, 0.31 mmol, 1.0 eq.) in 10 mL dry DCM and DIPEA (0.29 mL, 1.8 mmol, 5 eq.) was cooled to 0 °C. Methyl *N*,*N*-diisopropylchlorophosphoramidite (0.122 mL, 0.63 mmol, 2.0 eq.) was slowly added drop by drop. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. It was then quenched by addition of saturated aqueous $NaHCO_3$ solution and extracted with DCM. The organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure (water bath at 30 °C). The residue war purified by chromatography (SiO_2, EtOAc/cyclohexane 2:1 + 2% Et_3N). Lyophilization of the product fractions from benzene yielded 350 mg (89%) of phosphoramidite **3** as a colorless amorphous powder. ^{1}H-NMR (400 MHz, C_6D_6): 8.38 (d, *J* = 1.5 Hz, 1H), 8.00 (dd, *J* = 7.9, 1.3 Hz, 2H), 7.83 – 7.74 (m, 6H), 7.46 (dd, *J* = 7.7, 1.3 Hz, 2H), 7.40 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.02 (td, *J* = 7.6, 1.3 Hz, 2H), 6.96 (td, *J* = 7.7, 1.3 Hz, 2H), 6.93 – 6.90 (m, 1H), 6.88 (t, *J* = 5.5 Hz, 2H), 6.55 (d, *J* = 8.2 Hz, 4H), 6.50 (d, *J* = 8.1 Hz, 2H), 3.75 – 3.68 (m, 1H), 3.67 – 3.60 (m, 2H), 3.57 – 3.47 (m, 6H), 3.36 (d, *J* = 12.8 Hz, 3H), 2.59 – 2.49 (m, 6H), 2.27 (t, *J* = 7.0 Hz, 2H), 1.61 (s, 6H), 1.60 (s, 3H), 1.38 – 1.27 (m, 8H), 1.22 (d, *J* = 6.7 Hz, 6H), 1.19 (d, *J* = 6.8 Hz, 6H) ppm. ^{31}P-NMR (162 MHz, C_6D_6): 148.11 ppm.

Hydrogenation of the alkyne side chain of compound 22



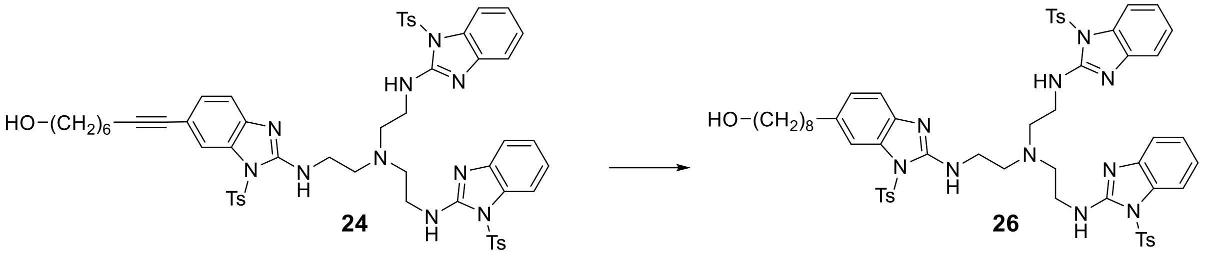
One table spoon of pre-activated Raney-Ni 2800 (Sigma-Aldrich) was added to a solution of alkyne **22** (300 mg, 285 mmol) in 2 mL THF and 15 mL EtOH. The suspension was intensely stirred under an atmosphere of hydrogen at room temperature overnight. It was then filtered over celite and washed with DCM. The solvent was removed under reduced pressure and the residue was lyophilized from benzene to yield a colorless amorphous powder of **25** (130 mg, 43%). ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): 7.87 – 7.82 (m, 6H), 7.65 (dd, *J* = 8.2, 1.2 Hz, 2H), 7.46 (d, *J* = 1.6 Hz, 1H), 7.27 – 7.22 (m, 6H), 7.18 – 7.15 (m, 4H), 7.12 – 7.07 (m, 3H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.02 (td, *J* = 7.7, 1.3 Hz, 2H), 6.93 (dd, *J* = 8.1, 1.7 Hz, 1H), 4.32 (t, *J* = 5.1 Hz, 1H), 3.66 – 3.59 (m, 6H), 3.38 (td, *J* = 6.5, 5.1 Hz, 2H), 2.94 (t, *J* = 6.9 Hz, 6H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.14 (s, 9H), 1.55 (quintet, *J* = 7.5 Hz, 2H), 1.40 (quintet, *J* = 6.7 Hz, 2H), 1.34 – 1.27 (m, 2H), 1.27 – 1.20 (m, 2H) ppm. ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): 151.95, 151.74, 146.13, 146.06, 142.72, 140.74, 135.14, 133.50, 133.47, 130.45, 130.40, 130.21, 130.14, 126.56, 126.54, 124.90, 124.68, 120.71, 116.20, 115.81, 112.17, 111.97, 60.68, 52.34, 40.90, 35.12, 32.50, 31.55, 28.31, 25.33, 20.85 ppm. MS (ESI+): *m/z* calcd. for $C_{54}H_{61}N_{10}O_7S_3$ [M+H$^+$]: 1057.39; found 1057.45. HRMS (ESI-TOF): *m/z* calcd. for $C_{54}H_{62}N_{10}O_7S_3$ [M+2H$^+$]: 529.1983; found 529.1972.

Phosphoramidite 4



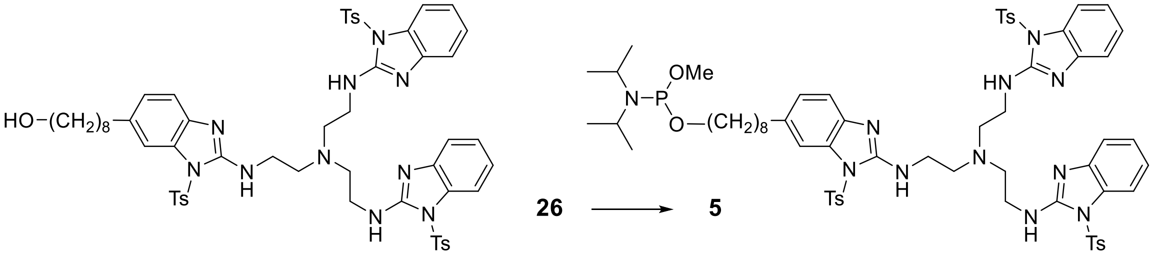
A solution of compound **25** (110 mg, 0.104 mmol, 1.0 eq.) in 5 mL dry DCM and DIPEA (0.09 mL, 0.5 mmol, 5 eq.) was cooled to 0 °C. Methyl *N*,*N*-diisopropylchlorophosphoramidite (0.039 mL, 0.200 mmol, 1.9 eq.) was slowly added dropwise. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. It was then quenched by addition of saturated aqueous $NaHCO_3$ solution and extracted with DCM. The organic phases were dried with MgSO4 and the solvent was removed under reduced pressure (water bath at 30 °C). The residue war purified by chromatography (SiO_2, EtOAc/cyclohexane 2:1 + 2% Et_3N). Lyophilization of the product fractions from benzene yielded 60 mg (47%) of phosphoramidite **4** as a colorless amorphous powder. ^{1}H-NMR (400 MHz, C_6D_6): 8.03 – 7.99 (m, 2H), 7.96 (d, *J* = 1.6 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.84 – 7.78 (m, 4H), 7.47 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.05 – 7.00 (m, 2H), 6.96 (td, *J* = 7.7, 1.4 Hz, 2H), 6.93 – 6.83 (m, 4H), 6.62 (d, *J* = 8.3 Hz, 2H), 6.59 – 6.53 (m, 4H), 3.80 – 3.70 (m, 1H), 3.70 – 3.60 (m, 2H), 3.59 – 3.50 (m, 6H), 3.36 (d, *J* = 12.9 Hz, 3H), 2.61 – 2.52 (m, 8H), 1.65 (s, 3H), 1.62 (s, 6H), 1.60 – 1.55 (m, 2H), 1.41 – 1.30 (m, 4H), 1.30 – 1.25 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 6H), 1.19 (d, *J* = 6.8 Hz, 6H) ppm. ^{31}P-NMR (162 MHz, C_6D_6): 148.10 ppm.

Hydrogenation of the alkyne side chain of compound 24



Alkyne **24** (340 mg, 0,314 mmol) was dissolved in 2 mL THF and 15 mL EtOH. 34 mg of 10% Pd/C were added and the suspension was intensely stirred under an atmosphere of hydrogen at room temperature overnight. According to NMR, the reaction was still incomplete and was repeated as described. Afterwards, the suspension was filtered over celite, washed and the solvent evaporated. The residue was lyophilized from benzene to yield a colorless amorphous powder of **26** (340 mg, quant.). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.89 – 7.80 (m, 6H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 1.6 Hz, 1H), 7.30 – 7.20 (m, 6H), 7.19 – 6.99 (m, 10H), 6.93 (dd, *J* = 8.1, 1.6 Hz, 1H), 4.31 (t, *J* = 5.1 Hz, 1H), 3.67 – 3.57 (m, 6H), 3.37 (td, *J* = 6.5, 5.1 Hz, 2H), 2.93 (t, *J* = 6.8 Hz, 6H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.15 (s, 9H), 1.59 – 1.49 (m, 2H), 1.45 – 1.35 (m, 2H), 1.31 – 1.20 (m, 8H) ppm. ^{13}C-NMR (101 MHz, DMSO-*d*$_6$): 151.97, 151.76, 146.21, 146.12, 142.74, 140.76, 135.17, 133.50, 133.45, 130.46, 130.41, 130.28, 130.18, 126.26, 126.59, 124.96, 124.74, 120.77, 116.23, 115.83, 112.21, 112.01, 60.71, 52.32, 35.17, 32.55, 31.57, 28.98, 28.44, 25.52, 20.91 ppm. MS (ESI+): *m/z* calcd. for $C_{56}H_{65}N_{10}O_7S_3$ [M+H$^+$]: 1085.42; found 1085.56. HRMS (ESI-TOF): *m/z* calcd. for $C_{56}H_{66}N_{10}O_7S_3$ [M+2H$^+$]: 543.2134; found 543.2134.

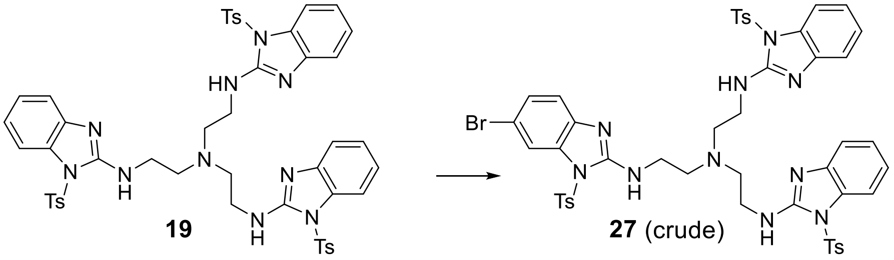
Phosphoramidite 5



A solution of compound **26** (170 mg, 0.157 mmol, 1.0 eq.) in 6 mL dry DCM and DIPEA (0.17 mL, 1 mmol, 6.4 eq.) was cooled to 0 °C. Methyl *N*,*N*-diisopropylchlorophosphoramidite (0.097 mL, 0.50 mmol, 3.2 eq.) was slowly added drop by drop. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. It was then quenched by addition of saturated aqueous $NaHCO_3$ solution and extracted with DCM. The organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure (water bath at 30 °C). The residue war purified by chromatography (SiO_2, EtOAc/cyclohexane 2:1 + 2% Et_3N). Lyophilization of the product fractions from benzene yielded 130 mg (67%) of phosphoramidite **5** as a colorless amorphous powder. ^{1}H-NMR (400 MHz, C_6D_6): 8.01 (dd, *J* = 7.9, 1.3 Hz, 2H), 7.99 (d, *J* = 1.7 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 8.4 Hz, 4H), 7.49 – 7.43 (m, 3H), 7.03 (td, *J* = 7.6, 1.4 Hz, 2H), 6.99 – 6.92 (m, 3H), 6.90 (t, *J* = 5.5 Hz, 2H), 6.85 (t, *J* = 5.6 Hz, 1H), 6.61 (d, *J* = 8.1 Hz, 2H), 6.57 (d, *J* = 8.2 Hz, 4H), 3.81 – 3.71 (m, 1H), 3.71 – 3.61 (m, 2H), 3.59 – 3.51 (m, 6H), 3.37 (d, *J* = 12.8 Hz, 3H), 2.64 – 2.52 (m, 8H), 1.67 – 1.55 (m, 4H), 1.64 (s, 3H), 1.62 (s, 6H), 1.43 – 1.35 (m, 2H), 1.28 – 1.24 (m, 6H), 1.22 (d, *J* = 6.8 Hz, 6H), 1.19 (d, *J* = 6.8 Hz, 6H) ppm. ^{31}P-NMR (162 MHz, C_6D_6): 148.14 ppm.

Synthesis of phosphoramidites 4 and 6 via statistical bromination

Bromotrisbenzimidazole 27



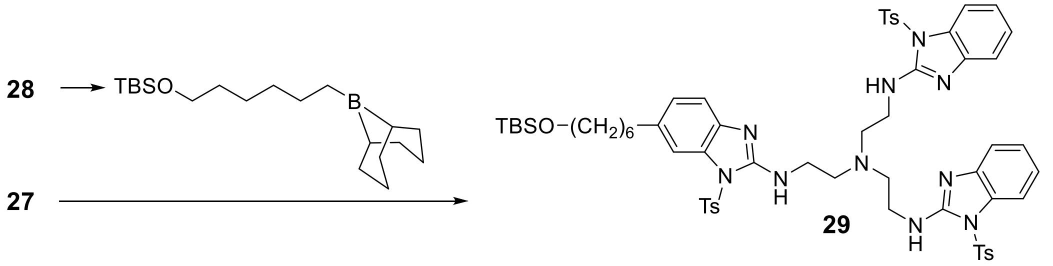
Compound **19** (5.26 g, 5.50 mmol, 1 eq.) was dissolved in 50 mL of HOAc. After addition of *N*-bromosuccinimide (0.98 g, 5.50 mmol, 1 eq.), the solution was stirred at room temperature overnight. After that it was diluted with EtOAc, crushed ice was added and 50 mL of 30% aqueous NaOH solution. The aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ solution (until gas evolution had stopped), washed with brine and dried with $MgSO_4$. Evaporation of the solvent yielded a crude product containing compound **27** as a brownish foam, directly used in the next step without purification. MS (ESI+): *m/z* = calcd. for $C_{48}H_{48}BrN_{10}O_6S_3$ [M+H$^+$]: 1035.21; found 1035.09.

Silylation of 5-hexen-1-ol yielding compound 28 [17]

$$\mathrm{TBSO{-}(CH_2)_4{-}CH{=}CH_2} \quad \mathbf{28}$$

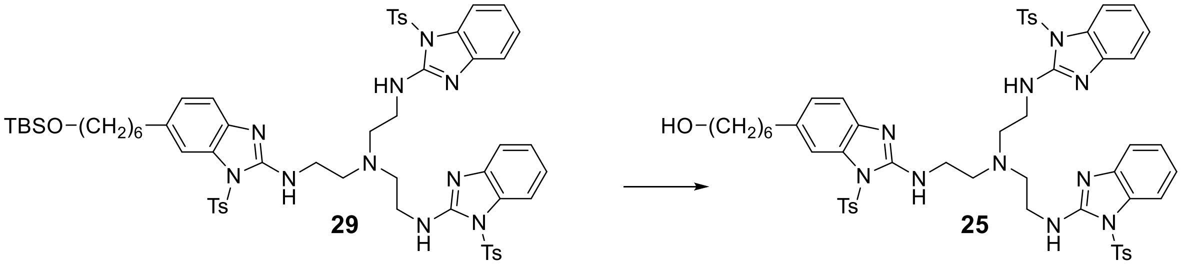
To a solution of 5-hexen-1-ol (6.69 g, 66.8 mmol, 1 eq.) in 80 mL DMF were added at 0 °C imidazole (5.00, 73.5 mmol, 1.1 eq.) and TBS-Cl (11.1 g, 73.5 mmol, 1.1 eq.). The mixture was stirred for 1 h at room temperature, then quenched by addition of aqueous NH_4Cl solution and extracted with Et_2O. The combined organic phases were washed with water and with brine and were dried with $MgSO_4$. Evaporation of the solvent at reduced pressure yielded 14.27 g (quant.) of **28** as a colorless oil which was used in the next step without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1 H), 5.00 (ddt, *J* = 17.1, 2.2, 1.6 Hz, 1H), 4.94 (ddt, *J* = 10.2, 2,3, 1.2 Hz, 1H), 3.61 (t, *J* = 6.4 Hz, 2H), 2.10 – 2.03 (m, 2H), 1.57 – 1.48 (m, 2H), 1.48 – 1.38 (m, 2H), 0.89 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 139.11, 114.50, 63.23, 33.69, 32.46, 26.13, 25.31, 18.53, -5.12 ppm. ^{29}Si-NMR (80 MHz, $CDCl_3$): 18.43 ppm. The ^{1}H and ^{13}C NMR spectra are consistent with data from ref. 17.

Suzuki reaction of 27 forming compound 29



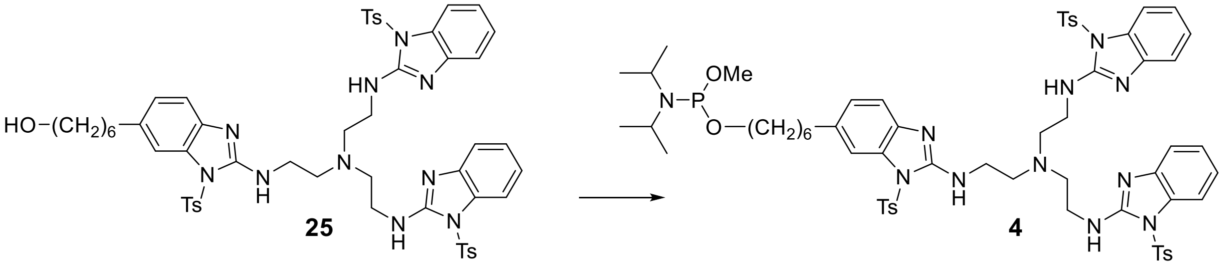
The crude mixture containing compound **27** (from 5.26 g **19**, 5.50 mmol, 1 eq.), Pd SPhos G3 (210 mg, 0.27 mmol, 5 mol %), SPhos (110 mg, 0.27 mmol, 5 mol %) and K_3PO_4 (2.29 g, 10.8 mmol, 2 eq.) were placed in a flask filled with argon. In a second flask, a THF solution of 9-BBN (0.5 M, 21.6 mL, 2 eq.) was added dropwise at 0 °C to a solution of hexenol **28** (2.32 g, 10.81 mmol, 2 eq.) in 21 mL of THF. To complete the hydroboration, the solution was stirred for 1 h at 0 °C and for 2 h at room temperature. 20 mL of THF were added, the solution thoroughly purged with argon and transferred to the first flask containing **27**, catalyst and base. 10 mL of degassed water was added and the mixture was stirred at 60 °C for 1.5 h. After that the solvent was evaporated, the residue diluted with water and extracted with EtOAc. Drying with $MgSO_4$ and evaporation of the solvent yielded a crude product that was enriched by chromatography (silica, EtOAc/cyclohexane 2:1). Some pure fractions were used for characterization. Most of the material, however, was a mixture of **29** with analogs containing none (**19**) or two silylated side chains. After removal of the solvent, crude product **29** was obtained as a colorless foam and directly used in desilylation step. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.82 – 7.77 (m, 6H), 7.68 (dd, *J* = 8.1, 1.2 Hz, 2H), 7.51 (d, *J* = 1.6 Hz, 1H), 7.29 (dd, *J* = 7.9, 1.2 Hz, 2H), 7.20 – 7.11 (m, 9H), 7.03 (td, *J* = 7.7, 1.2 Hz, 2H), 6.96 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.88 (t, *J* = 5.4 Hz, 2H), 6.80 (t, *J* = 5.5 Hz, 1H), 3.74 – 3.67 (m, 6H), 3.61 (t, *J* = 6.6 Hz, 2H), 3.02 (t, *J* = 6.1 Hz, 6H), 2.65 (t, *J* = 7.6 Hz, 2H), 2.26 (s, 3H), 2.25 (s, 6H), 1.62 (quintet, *J* = 7.4 Hz, 2H), 1.52 (quintet, *J* = 6.7 Hz, 2H), 1.39 – 1.30 (m, 4H), 0.90 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.24, 152.00, 145.94, 145.86, 142.76, 140.73, 136.33, 134.61, 134.55, 130.20, 130.14, 126.99, 126.95, 125.15, 124.81, 121.26, 116.88, 116.41, 112.51, 112.41, 63.39, 54.15, 41.80, 36.17, 32.98, 32.22, 29.12, 26.11, 25.86, 21.65, 18.48, -5.13 ppm. MS (ESI+): *m/z* calcd. for $C_{60}H_{75}N_{10}O_7S_3Si$ [M+H$^+$]: 1171.47; found 1171.64. HRMS (ESI-TOF): *m/z* calcd. for $C_{60}H_{76}N_{10}O_7S_3Si$ [M+2H$^+$]: 586.2415; found 586.2406.

Desilyation of 29 forming alcohol 25



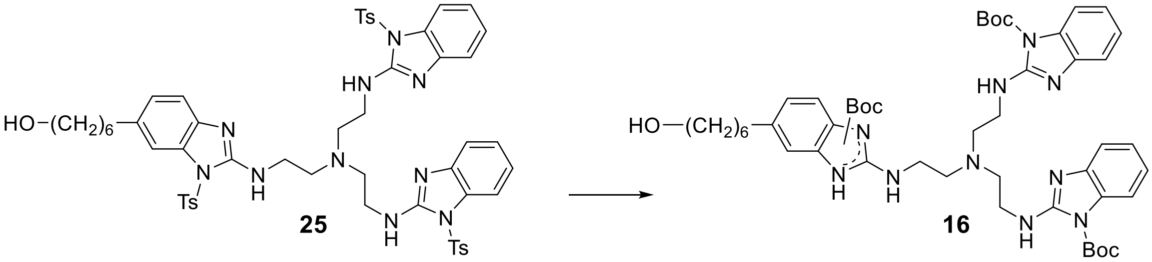
The TBS protected compound **29** (see above) was dissolved in 10 mL of THF. 30 mL HOAc and 10 mL H_2O were added and the mixture was stirred overnight at room temperature. The solution was then diluted with EtOAc. Ice was added and 30 mL of 30% aqueous NaOH solution. After phase separation, the aqueous phase was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution until gas evolution had stopped, were washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by chromatography (silica, DMC/MeOH 30:1). Pure compound **25** was obtained as a colorless foam (2.03 g, 35% yield over 3 steps from compound **19**). The ^{1}H- and ^{13}C-NMR spectra matched the data reported above for compound **25**. MS (ESI+): *m/z* calcd. for $C_{54}H_{61}N_{10}O_7S_3$ [M+H$^+$]: 1057.39; found 1057.45. HRMS (ESI-TOF): *m/z* calcd. for $C_{54}H_{62}N_{10}O_7S_3$ [M+2H$^+$]: 529.1983; found 529.1972.

Phosphoramidite 4



To a solution of compound **25** (1.33 g, 1.26 mmol, 1 eq.) in 20 mL DCM was added 4,5-dicyanoimidazole (163 mg, 1.38 mmol, 1.1 eq.) and methyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (495 mg, 1.89 mmol, 1.5 eq.). The solution was stirred at room temperature overnight. Afterwards saturated aqueous $NaHCO_3$ solution was added. The water phase was extracted with DCM and the combined organic phases were dried with $MgSO_4$. The solvent was evaporated at reduced pressure (water bath at 30 °C) and the residue purified by chromatography (SiO_2, EtOAc/cyclohexane 2:1 + 2% Et_3N). Product fractions were concentrated and finally lyophilized from benzene to obtain phosphoramidite **4** as an amorphous colorless powder (700 mg, 46%). The ^{1}H- and ^{31}P-NMR spectra matched the data reported above for compound **4**.

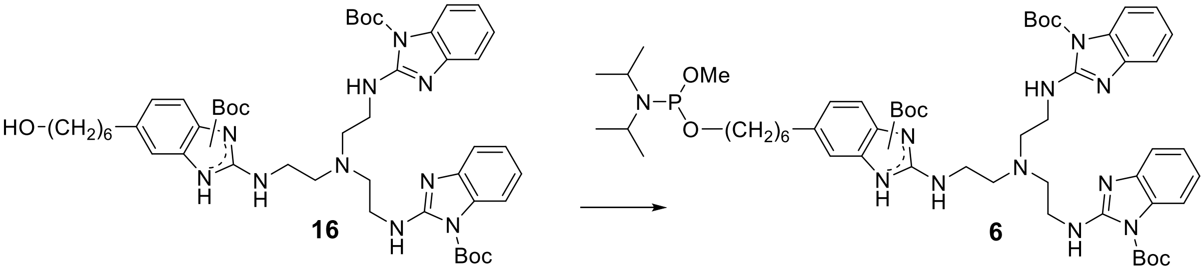
Conversion of 25 into the Boc derivative 16



The tosylated compound **25** (2.03 g, 1.92 mmol, 1 eq.) was dissolved in 10 mL of THF. After addition of 20 mL MeOH, 7 mL H_2O and 7 mL 30% aqueous NaOH solution, the mixture was stirred at room temperature for about 3 h. TLC (DCM/MeOH 10:1 +2% NH_3) should show the absence of partially sulfonylated material. Brine was added, phases separated and the aqueous phase was extracted several times with DCM and once with EtOAc. Drying of the combined organic phases with $MgSO_4$ and removal of the solvent provided the detosylated trisbenzimidazole **15**. MS (ESI+): *m/z* calcd. for $C_{33}H_{43}N_{10}O$ [M+H$^+$]: 595.36; found 595.30.

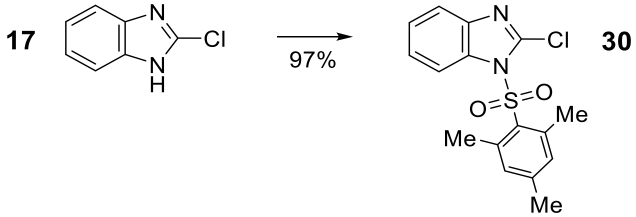
The residue of crude compound **15** was dissolved in 30 mL DMF. Triethylamine (1.06 mL, 7.68 mmol, 4 eq.) and Boc_2O (1.47 g, 6.72 mmol, 3.5 eq.) were added and the mixture was stirred overnight at room temperature. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (silica, DCM/MeOH 20:1) to yield 1.05 g of compound **16** (61%) as a colorless foam. ^{1}H-NMR (600 MHz, DMSO-d$_6$): 7.54 (q, *J* = 5.4 Hz, 2H), 7.51 (ddd, *J* = 8.1, 3.6, 1.1 Hz, 2H), 7.46 (t, *J* = 5.5 Hz, 0.5H), 7.37 (d, *J* = 8.2 Hz, 0.5H), 7.36 (d, *J* = 1.6 Hz, 0.5H), 7.19 (ddd, *J* = 7.9, 2.9, 1.2 Hz, 2H), 7.10 (tt, *J* = 7.7, 1.3 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 0.5H), 6.99 – 6.95 (m, 2.5H), 6.92 (dd, *J* = 8.0, 1.7 Hz, 0.5H), 6.78 (dd, *J* = 8.2, 1.7 Hz, 0.5H), 4.31 (t, *J* = 5.1 Hz, 1H), 3.57 – 3.48 (m, 6H), 3.36 (td, *J* = 6.5, 5.1 Hz, 2H), 2.88 – 2.82 (m, 6H), 2.59 (t, *J* = 7.5 Hz, 1H), 2.54 (t, *J* = 7.5 Hz, 1H), 1.61 – 1.51 (m, 2H), 1.50 – 1.44 (m, 27H), 1.42 – 1.36 (m, 2H), 1.32 – 1.23 (m, 4H) ppm (mixture of two isomers, the spectrum matched the data reported above for compound **16**). ^{13}C-NMR (151 MHz, DMSO-d$_6$): 153.69, 153.60, 153.37, 150.11, 142.82, 142.72, 140.74, 138.04, 133.74, 130.40, 130.30, 128.35, 124.01, 123.82, 119.97, 119.76, 115.62, 115.41, 115.16, 113.59, 113.47, 113.14, 85.25, 85.06, 60.65, 60.62, 53.52, 53.47, 41.00, 35.19, 35.02, 32.47, 32.43, 31.34, 31.25, 28.37, 28.31, 27.33, 27.29, 26.83, 25.32, 25.29 ppm (mixture of two isomers, the spectrum matched the data reported above for compound **16**). MS (ESI): *m/z* calcd. for $C_{48}H_{67}N_{10}O_7$ [M+H$^+$]: 895.52; found 895.56. HRMS (ESI-TOF): *m/z* calcd. for $C_{48}H_{67}N_{10}O_7$ [M+H$^+$]: 895.51942; found 895.5227.

Phosphoramidite 6



A solution of compound **16** (1.05 g, 1.17 mmol, 1 eq.) in 20 mL DCM and DIPEA (1.00 mL, 5.87 mmol, 5 eq.) was cooled to 0 °C. Methyl *N*,*N*-diisopropylchlorophosphoramidite (464 mg, 2.34 mmol, 2 eq.) was added. After stirring for 30 min at 0 °C, the mixture was stirred for 1 h at room temperature. Addition of saturated aqueous $NaHCO_3$ solution quenched the reaction. The water phase was extracted with DCM and the combined organic phases were dried with $MgSO_4$. The solvent was evaporated at reduced pressure (water bath at 30 °C) and the residue purified by chromatography (silica, EtOAc/cyclohexane 2:1 + 2% Et_3N). Product fractions were concentrated and finally lyophilized from benzene to obtain phosphoramidite **6** as an amorphous colorless powder. Yield: 1.08 g (87%). ^{1}H-NMR (400 MHz, C_6D_6): 7.68 (t, *J* = 7.8 Hz, 4H), 7.64 – 7.59 (m, 4H), 7.55 (d, *J* = 1.6 Hz, 0.5H), 7.22 – 7.17 (m, 2.5H), 7.09 – 7.02 (m, 2.5H), 6.95 (dd, *J* = 8.2, 1.7 Hz, 0.5H), 3.79 – 3.70 (m, 1H), 3.68 – 3.59 (m, 8H), 3.36 (d, *J* = 12.8 Hz, 1.5H), 3.35 (d, *J* = 12.8 Hz, 1.5H), 2.71 (t, *J* = 7.6 Hz, 1H), 2.64 (t, *J* = 7.6 Hz, 1H), 2.51 (q, *J* = 5.5 Hz, 6H), 1.75 - 1.56 (m, 4H), 1.41 – 1.33 (m, 4H), 1.30 – 1.23 (m, 27H), 1.23 – 1.17 (m, 12H) ppm (mixture of two isomers). ^{31}P-NMR (162 MHz, C_6D_6): 148.17, 148.12 ppm (mixture of two isomers).

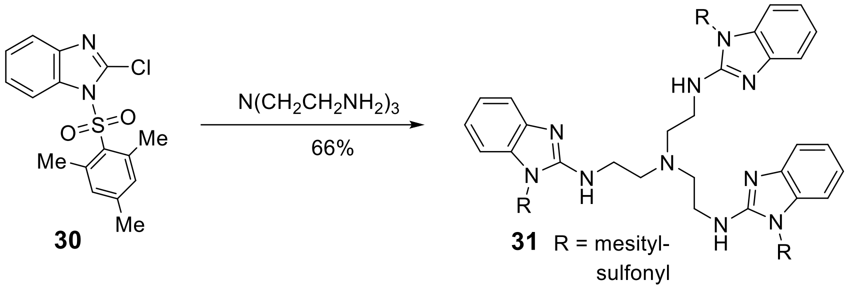
2-Chloro-1-mesitylsulfonyl-1*H*-benzo[*d*]imidazole 30



10 g of 2-chlorobenzimidazole **17** (65.5 mmol, 1 eq.) were suspended in 100 mL dry DCM. The stirred suspension was cooled to 0 °C, triethylamine (11.1 mL, 79.6 mmol, 1.2 eq.) was added and then 2,4,6-trimethylbenzenesulfonylchloride (14.3 g, 65.5 mmol, 1 eq., in small portions). The mixture was warmed up to room temperature. After about 30 min it became clear for a moment and started to separate a second phase. The mixture was stirred for two days at room temperature. It was transferred into a separation funnel and washed three times with 150 mL water and once with 150 mL of saturated aqueous NaCl solution. The aqueous phases were extracted with 150 mL DCM and the combined organic phases dried with $MgSO_4$. After evaporation of the solvent, the product crystallized. It was washed with hexanes and dried in vacuum to yield colorless crystals of **30** (21.2 g, 97%). Mp. 137-138 °C. ^{1}H-NMR (400 MHz, C_6D_6): 8.21 (d, *J* = 8.5 Hz, 1 H), 7.60 (ddd, *J* = 7.9, 1.4, 0.7 Hz, 1H), 7.06 (ddd, *J* = 8.4, 7.4, 1.4 Hz, 1H), 6.99 (ddd, *J* = 8.6, 7.4, 1.2 Hz, 1H), 6.33 (s, 2H), 2.30 (s, 6H),

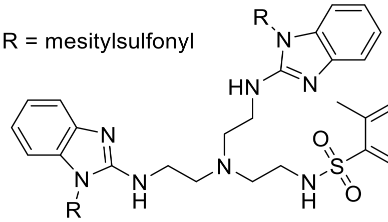
1.76 (s, 1.76) ppm. ^{13}C-NMR (101 MHz, C_6D_6): 144.74, 141.18, 140.88, 138.32, 135.80, 133.42, 132.37, 125.59, 124.85, 120.48, 114.75, 22.44, 20.73 ppm. MS (ESI): *m/z* calcd. for $C_{16}H_{16}ClN_2O_2S$ [M+H$^+$]: 335.06; found 334.94. HRMS (ESI-TOF): *m/z* calcd. for $C_{16}H_{16}ClN_2O_2S$ [M+H$^+$]: 335.06210; found 335.0620. Compound **30** is unstable in DMSO and can slowly decompose. Aged samples may give reduced yields in the subsequent step.

Mesitylsulfonylated trisbenzimidazole 31



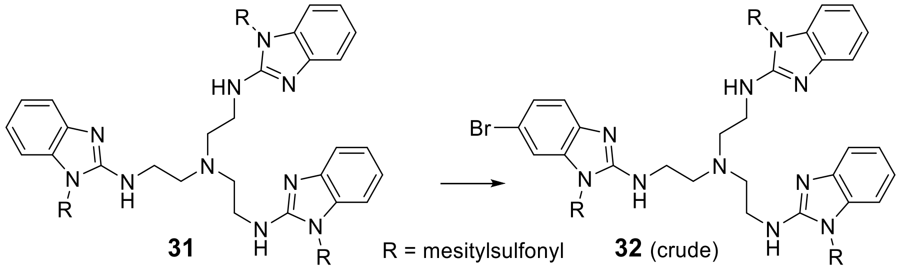
In a 100 mL single neck flask, 5.00 g (14.9 mmol, 3.4 eq.) freshly prepared benzimidazole **30** were dissolved with gentle heating in 20 mL of dry benzene. 5.40 g (16.5 mmol, 3.7 eq.) Cs_2CO_3 and 662 µL (4.42 mmol, 1 eq.) TREN were added. The white suspension was heated to reflux for 4.5 h until ninhydrin assays became negative. After evaporation of benzene, the residue was suspended in 300 mL DCM. It was washed with 200 mL and with 100 mL water and with 100 mL of brine. The aqueous phases were reextracted with 100 mL DCM. After drying the combined DCM phases with $MgSO_4$, the solvent was removed and the residue was purified by chromatography on 200 g silica gel with ethyl acetate/hexanes 5:1. After elution of byproduct **S6**, the solvent was changed to ethyl acetate/hexanes 10:1. Evaporation of the pure fractions yielded 3.02 g (66%) of compound **31** as a colorless foam. **31**: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 7.24 (dt, *J* = 7.8, 1.0 Hz, 3H), 7.08 – 7.04 (m, 12H), 6.84 (td, *J* = 7.7, 1.2 Hz, 3H), 6.72 (dt, *J* = 7.9, 0.9 Hz, 3H), 3.64 (q, *J* = 6.3 Hz, 6H), 2.91 (t, *J* = 6.8 Hz, 6H), 2.41 (s, 18H), 2.22 (s, 9H) ppm. The sample contained traces of EtOAc. ^{13}C-NMR (101 MHz, DMSO-*d*$_6$): 152.45, 144.71, 141.89, 139.32, 132.38, 131.80, 130.20, 124.02, 120.32, 116.27, 110.12, 52.46, 40.94, 21.47, 20.74, ppm. The sample contained traces of EtOAc. MS (ESI): *m/z* calcd. for $C_{54}H_{61}N_{10}O_6S_3$ [M+H$^+$]: 1041.39; found 1041.45. HRMS (ESI-TOF): *m/z* calcd. for $C_{54}H_{61}N_{10}O_6S_3$ [M+H$^+$]: 1041.39377; found 1041.3943.

S6 R = mesitylsulfonyl



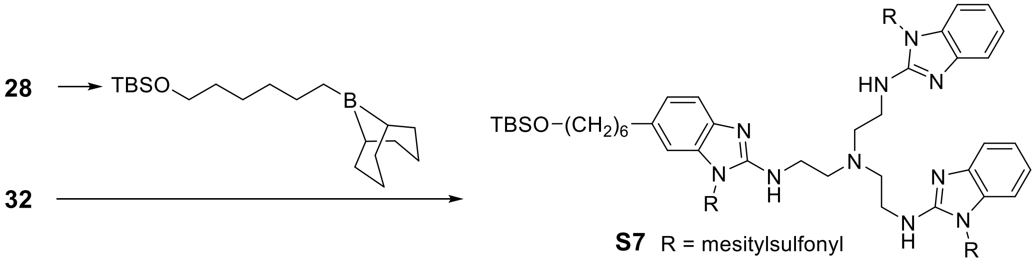
S6: ^{1}H-NMR (400 MHz, CDCl3): 7.32 (ddd, *J* = 7.9, 1.1, 0.6 Hz, 2H), 7.08 (td, *J* = 7.7, 1.1 Hz, 2H), 6.92 (s, 4H), 6.85 (s, 2H), 6.84 – 6.79 (m, 4H), 6.70 (ddd, *J* = 8.0, 1.1, 0.6 Hz, 2H), 3.65 (q, *J* = 6.1 Hz, 4H), 3.11 (q, *J* = 6.2 Hz, 2H), 2.87 (t, *J* = 6.4 Hz, 4H), 2.78 (t, *J* = 5.6 Hz, 2H), 2.60 (s, 6H), 2.48 (s, 12H), 2.27 (s, 6H), 2.20 (s, 3H) ppm. The sample contained traces of EtOAc. MS (ESI): *m/z* calcd. for $C_{47}H_{57}N_8O_6S_3$ [M+H$^+$]: 925.36; found 925.37. HRMS (ESI-TOF): *m/z* calcd. for $C_{47}H_{57}N_8O_6S_3$ [M+H$^+$]: 925.35632; found 925.3575.

Bromotrisbenzimidazole 32



Compound **31** (2.90 g, 2.79 mmol, 1 eq.) was dissolved in 50 mL of HOAc and *N*-bromosuccinimide (0.546 g, 3.07 mmol, 1.1 eq.) was added in small portions. The resulting yellowish solution was stirred at room temperature for 30 h (extending the reaction time improves the yield whereas using more NBS has the opposite effect). For workup, some ice and 120 mL ethyl acetate were added and 23 mL of ice-cold 30% aqueous NaOH solution. After shaking and phase separation, the aqueous phase was extracted twice with 120 mL ethyl acetate. The combined organic phases were washed three times with conc. aqueous $NaHCO_3$ until gas evolution had ceased, washed with brine and dried with $MgSO_4$. After evaporation of the solvent, 2.96 g (95%) of crude compound **32** remained as a colorless foam. It was used in the next step without further purification.

Suzuki reaction of 32 forming compound S7

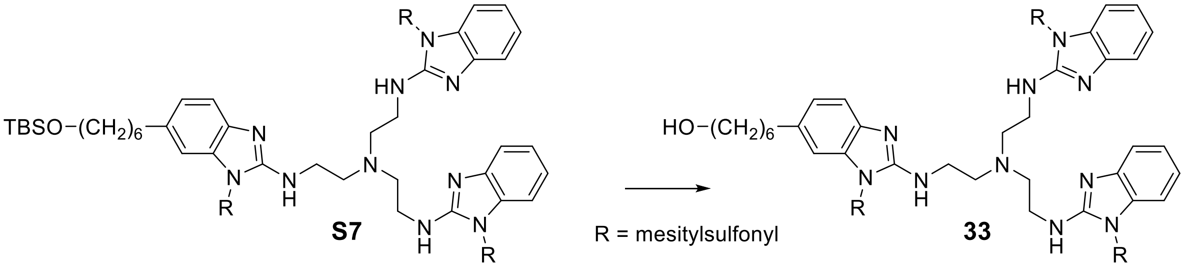


In a dry 50 mL three neck flask, 1.133 g of silylated hexenol **28** (5.28 mmol, 1.9 eq.) was dissolved in 10 mL dry THF at 0 °C under argon. 10.65 mL of a 0.5 M 9-BBN solution in THF was added dropwise (5.32 mmol, 1.9 eq.). The clear solution was stirred for 1 h at 0 °C and for 2 h at room temperature. TLC control (ethyl acetate/hexanes 1:9 + 1% triethylamine, staining with $KMnO_4$) showed complete hydroboration of **28**. The mixture was then diluted with 10 mL of THF and purged with argon. Meanwhile, a slow stream of argon was bubbled through Millipore-water for 1 h to remove oxygen. The crude compound **32** (from 2.90 g **31**, 2.79 mmol, 1 eq.) was placed under argon in a 100 mL flask with reflux condenser together with 0.102 g Pd SPhos G3 (0.132 mmol, 5 mol %), 0.056 g SPhos (0.132 mmol, 5 mol %) and 1.12 g K_3PO_4 (5.28 mmol, 1.9 eq.). The reflux condenser was briefly removed in a gentle stream of argon to add the hydroboration mixture (syringe) and 5.1 mL of degassed Millipore-water. The mixture was then heated under argon to 60 °C for 1.5 h and developed an orange color. After stirring at room temperature overnight, TLC (ethyl acetate + 1% triethylamine) showed the presence of products with one, two, or three side chains. The solvent was removed. The residue was distributed between 80 mL water and 140 mL ethyl acetate. The aqueous phase was extracted again with 140 and 100 mL ethyl acetate and the combined organic phases were washed with 140 mL of brine. After drying with $MgSO_4$ and removal of the solvent, 4.89 g of a product mixture was obtained as an orange foam. Chromatography on 250 g silica with ethyl acetate/hexanes 3:1 yielded 0.135 g (3%) of the trisalkyl derivative, 0.517 g (13%) of the bisalkyl derivative **S8** and 1.164 g (33%) of the monoalkyl product **S7**. By further elution of the column unreacted starting material **31** can be reisolated.

S7: ^{1}H-NMR (400 MHz, $CDCl_3$): 7.33 (ddd, *J* = 7.9, 1.1, 0.6 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.07 (ddd, *J* = 8.0, 7.8, 1.2 Hz, 2H), 6.89 (s, 6H), 6.87 – 6.84 (m, 2H), 6.81 (ddd, *J* = 8.5, 7.5, 1.2 Hz, 2H), 6.75 (t, *J* = 5.3 Hz, 1H, NH), 6.72 (d, *J* = 8.0 Hz, 2H), 6.56, (d, *J* = 1.1 Hz, 1H), 3.75 (m, 6H), 3.57 (t, *J* = 6.6 Hz, 2H) 3.03 (t, *J* = 6.5 Hz, 6H), 2.49 (s, 6H), 2.48 (s, 12H), 2.46 (t, *J* = 7.8, 2H), 2.24 (s, 9H), 1.48 – 1.37 (m, 4H), 1.29 – 1.12 (m, 4H), 0.89 (s, 9H), 0.04 (s, 6H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.77, 144.36, 144.28, 142.03, 140.04, 139.98, 132.54, 132.41, 132.30, 130.58, 124.31, 123.94, 120.47, 116.69, 116.18, 110.42, 110.26, 63.22, 53.48, 41.34, 35.74, 32.70, 31.37, 28.75, 25.96, 25.64, 22.23, 21.02, -5.29 ppm. MS (ESI): *m/z* calcd. for $C_{66}H_{87}N_{10}O_7S_3Si$ [M+H$^+$]: 1255.57; found 1255.59. HRMS (ESI-TOF): *m/z* calcd. for $C_{66}H_{87}N_{10}O_7S_3Si$ [M+H$^+$]: 1255.56906; found 1255.5719.

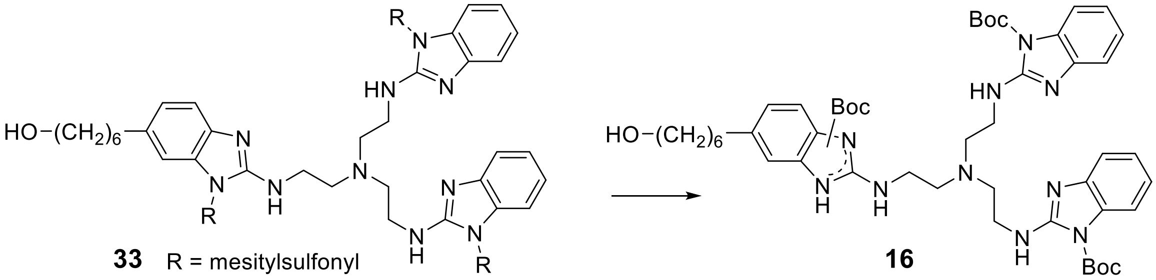
S8 (Bisalkyl): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.33 (ddd, *J* = 7.9, 1.1, 0.6 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 2H), 7.07 (ddd, *J* = 8.0, 7.8, 1.2 Hz, 1H), 6.89 (s, 6H), 6.85 (m, 3H), 6.81 (ddd, J = 8.5, 6.8, 1.8 Hz, 1H), 6.73 (m, 3H), 6.56 (m, 2H), 3.75 (m, 6H), 3.57 (t, *J* = 6.6 Hz, 4H), 3.02 (m, 6H), 2.49 (s, 12 H), 2.48 (s, 6H), 2.46 (t, J = 7.8 Hz, 4H), 2.24 (s, 9H), 1.49 – 1.36 (m, 8H), 1.29 – 1.12 (m, 8H), 0.89 (s, 18H), 0.04 (s, 12H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.89, 152.67, 144.64, 144.38, 142.14, 140.17, 140.10, 140.04, 135.32, 132.76, 132.67, 132.52, 132.42, 130.81, 130.70, 124.42, 124.05, 120.59, 116.80, 116.29, 110.55, 110.38, 63.34, 53.59, 41.48, 41.44, 35.87, 32.83, 31.50, 28.88, 26.09, 25.77, 22.36, 21.13, 18.48, -5.16 ppm.

Desilyation of S7 forming alcohol 33



The TBS protected compound **S7** (1.162 g, 0.925 mmol) was dissolved in 2.5 mL of THF. 7.6 mL HOAc and 2.5 mL water were added at room temperature. The turbid mixture later became clear. It was stirred at room temperature for 21 h and then diluted with 40 mL ethyl acetate. After addition of ice, the mixture was shaken with 7.5 mL cold 30% aqueous NaOH. The aqueous phase was extracted three times with 40 mL ethyl acetate. The combined organic phases were washed with $NaHCO_3$ solution until gas evolution had ceased, washed again with brine and were dried with $MgSO_4$. Solvent removal yielded 1.128 g of a colorless foam which was purified by chromatography on 75 g silica with ethyl acetate. 0.879 g (83%) of pure compound **33** could be isolated. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.32 (dt, *J* = 7.9, 0.9 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.07 (td, *J* = 7.7, 1.2 Hz, 2H), 6.89 (s, 6H), 6.88 – 6.77 (m, 5H), 6.77 – 6.70 (m, 3H), 6.57 (d, *J* = 1.5 Hz, 1H), 3.75 (m, 6H), 3.60 (t, *J* = 6.6 Hz, 2H), 3.03 (t, *J* = 6.6 Hz, 6H), 2.49 (s, 6H), 2.48 (s, 12H), 2.46 (t, *J* = 7.8 Hz, 2H), 2.24 (s, 9H), 1,79 (br. s, 1.5 H, OH + H_2O), 1.55 – 1.38 (m, 4H), 1.32 – 1.14 (m, 4H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$): 152.92, 152.71, 144.52, 144.43, 142.18, 140.18, 140.14, 140.11, 135.25, 132.79, 132.69, 132.57, 132.46, 130.87, 130.73, 124.47, 124.09, 120.63, 116.84, 116.36, 110.58, 110.46, 63.05, 53.65, 41.51, 35.84, 32.75, 31.45, 28.79, 25.67, 22.40, 21.18, 21.16 ppm. MS (ESI): *m/z* calcd. for $C_{60}H_{73}N_{10}O_7S_3$ [M+H$^+$]: 1141.48; found 1255.59. HRMS (ESI-TOF): *m/z* calcd. for $C_{60}H_{73}N_{10}O_7S_3$ [M+H$^+$]: 1141.48258; found 1141.4852.

Conversion of compound 33 into the Boc protected compound 16



Compound **33** (400 mg, 0.348 mmol, 1 eq.) was dissolved in 2.6 mL THF and 4.4 mL methanol. The flask was heated in a water bath to 60 °C. At this temperature, 1.4 mL of 30% aqueous NaOH was added and the mixture was stirred for exactly 18 min before it was cooled in an ice bath. TLC (methanol/DCM 1 : 10 + 2% aqueous NH_3) showed the absence of sulfonylated compounds. Minor amounts of byproducts are formed in this step. NaOH was then neutralized by addition of 1.4 mL HOAc. After evaporation of all solvents, the residue was well dried in vacuum to remove all traces of HOAc. The residue was suspended in 5.2 mL DMF and 0.210 mL DIPEA (1.22 mmol, 3.5 eq.) and stirred for 1 min. After addition of 0.299 mL Boc_2O (1.39 mmol, 4 eq.) the mixture was stirred at room temperature for 3 h until TLC (methanol/DCM 1 : 10 + 2% aqueous NH_3) indicated complete protection of the benzimidazoles. The mixture was diluted with 20 mL ethyl acetate and washed three times with saturated $NaHCO_3$ solution. The aqueous phases were reextracted with 20 mL ethyl acetate. Finally, the combined organic phases were washed with brine and dried with $MgSO_4$. The solvent was evaporated and the residue dried in vacuum to give 0.435 of a colorless foam. It was purified by chromatography on 40 g silica with methanol/DCM mixtures from 1:25 to 1:9. Pure fractions yielded 0.211 g (67%) of **16** as a colorless foam. The ^1H- and ^{13}C-NMR spectra (see Supporting Information) matched the data reported for compound **16** reported above. MS (ESI): *m/z* calcd. for $C_{48}H_{67}N_{10}O_7$ [M+H$^+$]: 895.52; found 895.55. HRMS (ESI-TOF): *m/z* calcd. for $C_{48}H_{67}N_{10}O_7$ [M+H$^+$]: 895.51942; found 895.5213.

General: Synthesis of oligonucleotides

Oligonucleotide chains were assembled (1 µmol scale) using an Expedite 8909 synthesizer (PerSeptive Biosystems). Phosphoramidites were purchased from Sigma-Aldrich (dA, dC, dG) and LGC Biosearch (LNA, dT), CPG supports from Sigma-Aldrich (dA, dG) and LGC Biosearch (dC, dT). TCA deblock (3% in DCM), Cap A (Ac_2O in THF), Cap B (NMI, THF, pyridine), oxidizer (I_2 in THF/pyridine/H_2O) and activator (0.3 M Hyacinth^{TM} 5-benzylthiotetrazole in MeCN) were obtained from emp Biotech. Acetonitrile diluent was purchased from Sigma-Aldrich. LGC Biosearch provided empty synthesis columns. These were manually charged with CPG support.

Manual conjugation of trisbenzimidazole phosphoramidites (conjugates 35 – 37) [3]

After chain assembly of the oligonucleotides (1 µmol scale, trityl-off), the dry CPG support was transferred into a 2 mL disposable syringe equipped with a UHMW-polyethylene filter (Porex Filtration Group, South Chesterfield, VA, USA). 10 mg of the corresponding phosphoramidite was added. The syringe was then dried in vacuum and filled with argon. The reactions started upon addition of 300 µL of a 0.25 M solution of 5-(ethylthio)-1*H*-tetrazole (in MeCN, Sigma-Aldrich). After shaking the syringe for 5 min, the solution was removed and discarded. The support was washed several times with MeCN. Then, the syringe was filled with 100 µL of oxidizer solution. After shaking for 30 s the solution was discarded and the support washed several times with MeCN.

Automated conjugation of trisbenzimidazole phosphoramidites (conjugates 37, 39, 40)

Tosyl-protected phosphoramidite **4** was dissolved in a 1:1 mixture of MeCN and DCM (0.1 M). The standard DNA coupling step of the synthesizer was used for attaching the catalyst.

Boc-protected phosphoramidite **6**: 1:1 mixture of MeCN and DCM (0.1 M). A modified coupling protocol was applied, see ref. 2. The standard protocol of the Expedite 8909 synthesizer reduced the coupling yield to roughly 25%.

Isolation and purification of conjugates

Tosyl-protected conjugates were cleaved from the support by incubation for 10 min with 1 mL AMA solution (32% aqueous ammonia/methylamine 1:1) at room temperature. The procedure was repeated once. To remove tosyl groups, the combined AMA solutions were heated for 3-5 h to 65 °C and then concentrated to dryness in a SpeedVac. This protocol is not compatible with LNA-C. Thus, conjugates containing LNA-C should be synthesized from the Boc-protected phosphoramidite **6** (or **1**). Cleavage from the support: incubation with 1 mL conc. aqueous ammonia (32%; 2 x 30 min). Removal of Boc: Incubation of the combined ammonia solutions at 55 °C overnight and drying in the SpeedVac. If the conjugates are prepared in the synthesizer, the support will be transferred into a polyethylene vial, incubated with AMA or ammonia (depending on the protective group) and heated as given above. Cleavage from the support and deprotection are thus combined.

For purification by RP-HPLC, a Jasco LC-900 system was used equipped with an UV-975 UV/Vis detector. Column: Phenomenex Jupiter 4 µm Proteo 90 Å (250 x 10 mm). Solvent A: MeOH. Solvent B: Aqueous hexafluoroisopropanol/Et_3N buffer (400 mM HFIP, 16 mM Et_3N, pH 7.9). Gradient: 5% A and 95% B from 0 to 2 min, 5% to 69% A and 95 to 31% B from 2 to 22 min. Column temperature: 60 °C. Product fractions were dried in a SpeedVac and dissolved in 500 µL of DEPC treated water.

Quantification

The concentration of oligonucleotides and of conjugates was determined by UV/VIS spectrometry and Lambert-Beer's law using a nanodrop2000 instrument. Extinction coefficients were estimated by the *nearest neighbor model*.

Mass spectrometry

Oligonucleotides and conjugates were characterized by LC-MS. A system from Thermo Fisher was used consisting of a *Vanquish Flex pump* and an *Orbitrap Exploris 120* mass spectrometer.

Analysis of RNA cleavage

Cy5-labeled RNAs **41** – **43** were obtained from Biospring (Frankfurt, Germany). Fragments after cleavage were separated by gel electrophoresis and quantified by fluorimetric detection (*ALFexpress II* DNA sequencer from *Amersham Biosciences*). Conditions: 1500 V (max.), 25 W (constant), gel temperature 55 °C, 2 s sampling interval, 400 min total running time. For data analysis the program AlleleLinks was used (*Amersham Biosciences*).

Rate saturation experiments

Cy5-labeled RNAs **41** – **43** (2 µL, 0.75 µM) and catalyst conjugates **35** – **40** (2 µL, 0.75 µM – 24 µM) were mixed in an Eppendorf vial (DNA LoBind) with Tris – HCl buffer (2 µL, pH 8.0, 250 mM) and 4 µL DEPC-treated H_2O. Final volume: 10 µL. Final concentrations: 150 nM RNA, 150 nM – 4.8 µM conjugate, 50 mM buffer. The solution was gently vortexed, centrifuged and then incubated for 16 h at 37 °C. The reactions were stopped by adding 15 µL of loading buffer (8 M urea, 20 mM EDTA, 0.2% crocein orange) and kept at 0 °C. For analysis, 1 µL of each sample was loaded onto the sequencer gel.

Reaction kinetics

Cy5-labeled RNAs **41** – **43** (10 µL, 0.75 µM) and catalyst conjugates **35** – **40** (10 µL, 6 µM) were mixed in an Eppendorf vial (DNA LoBind) with Tris – HCl buffer (10 µL, pH 8.0, 250 mM) and 20 µL DEPC-treated H_2O. Final volume: 50 µL. Final concentrations: 150 nM RNA, 1.2 µM conjugate, 50 mM buffer. The solution was gently vortexed, centrifuged and then incubated at 37 °C. At defined Intervals 5 µL aliquots were taken from this solution, quenched with 7.5 µL of loading buffer (8 M urea, 20 mM EDTA, 0.2% crocein orange) and stored in liquid nitrogen until analysis on the sequencer. For analysis, the samples were removed from the liquid nitrogen Dewar, thawed on ice and centrifuged. Afterwards 1 µL of this solution was loaded onto the gel.

RNA hydrolysis ladder

The RNA substrate of interest (375 nM) was dissolved in 20 µL of 0.25 M aqueous Na_2CO_3 (Eppendorf vial, DNA LoBind) and incubated for 12 min at 90 °C. The reaction was stopped by cooling to 0 °C and by addition of 5 µL 1M AcOH and of 37.5 µL loading buffer (8 M urea, 20 mM EDTA, 0.2% crocein orange). To localize the cleavage sites of conjugates, 5 µL of partially hydrolyzed RNA was mixed with 1 µL of the cleavage sample and loaded onto the gel (Figure 3, bottom).

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Supplementary Material

HPLC and mass spectra of conjugates, RNA cleavage data, NMR spectra.

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