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Photocaged ATP

Photocaged and Mixed Photocaged Bioreversible-Protected ATP Derivatives as Tools for the Controlled Release of ATP

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Dedicated to Prof. Dr. Jürgen Heck on the occasion of his 70th birthday

Abstract: Adenosine triphosphate (ATP) is known as the universal energy source for cellular processes, in addition, ATP also plays an important role in inflammation and cell signaling. Extracellular ATP binds to purinergic receptors and initiates further immune responses. To investigate these processes in-depth and to understand the complex mechanism of purinergic signaling, chemical tools are necessary. Here we present the synthesis of different photocaged ATP derivatives and the investigation of the light-induced release of ATP depending on the different synthesized photocleavable protecting groups based on the 2-nitrobenzyl moiety. Furthermore, we also present the synthesis of a mixed protected ATP-derivative as an example for a novel class of lipophilically caged nucleoside triphosphates. This new type of compounds is protected with a highly lipophilic non-toxic bio-removable acyloxybenzyl group and a photocleavable group. This combination may allow both passive cell uptake and controlled release of ATP by irradiation with non-harmful UV light.

Introduction

Adenosine triphosphate (ATP) is the universal energy source for all kinds of cellular processes and provides a defined amount of energy, that is stored in its phosphoanhydride bonds and released upon their cleavage. This energy is used for specific cellular processes like muscle contractions or biosynthesis of cellular components. Furthermore, ATP also plays an important role in inflammation and cell signaling.[1–5] In resting cells, the extracellular ATP concentration is rather low (0.4–1 mM), compared to the intracellular level (up to 10 mM).[6] It was observed that tissue damage, pathogens, or other cell stress led to a rapid increase in the level of extracellular ATP and that this extracellular ATP triggers a proinflammatory environment.[7–9] This led to further immune responses through signaling cascades, including the activation of immune T-cells or inflammation. ATP is released into the extracellular space by exocytosis of vesicles from the cell interior or by active transport via membrane-bound proteins. In the extracellular space, ATP binds to a wide range of P2 purinergic receptors, which are divided into two subsets: P2X and P2Y.[10,11] Both subsets are present on various kinds of immune cells, including macrophages, T cells, or neutrophils. P2X receptors are activated solely by ATP and mediate the influx or efflux of sodium, potassium, or calcium ions, while ATP activated P2Y receptors modulate the activation of various cellular enzymes.[12] The ATP-dependent activation of those P2 receptors promotes an inflammatory environment to fight pathogens or trigger tissue healing, for instance. To counteract the pro-inflammatory signaling and to cancel the immune response, the ectoenzymes CD39 and CD79 degrade ATP to adenosine, which is the natural extracellular counterpart of ATP.[13] An imbalance of this natural equilibrium between ATP and adenosine can lead to a chronic pro-inflammatory environment, which is considered to cause chronic inflammatory diseases.[13] For a better understanding of this complex purinergic system, chemical tools are crucial to investigate distinct processes.

Photoremovable protection groups are promising candidates for this kind of chemical tools.[14–16] By protecting appropriate compounds with those photoremovable groups the ability to release the protected substrate with exact control over time and space is gained. The application of this concept to ATP may enable the investigation of the effects triggered by extracellular ATP directly and further on it may be possible to observe initiated signaling events and immune responses.[17,18] Caged-ATP was first synthesized and described by Kaplan and co-workers, who protected the γ-phosphate with one photocleavable 2-nitrobenzyl group (NB-group). After photolytic cleavage of the NB-group Kaplan and co-workers could observe the activation of a Na:K ion pump of human red blood cell ghosts.[19] In contrast to the work of Kaplan and co-workers, we studied different kinds of electron donor substituted 2-nitrobenzyl moieties to shift the absorption maximum towards lower energy

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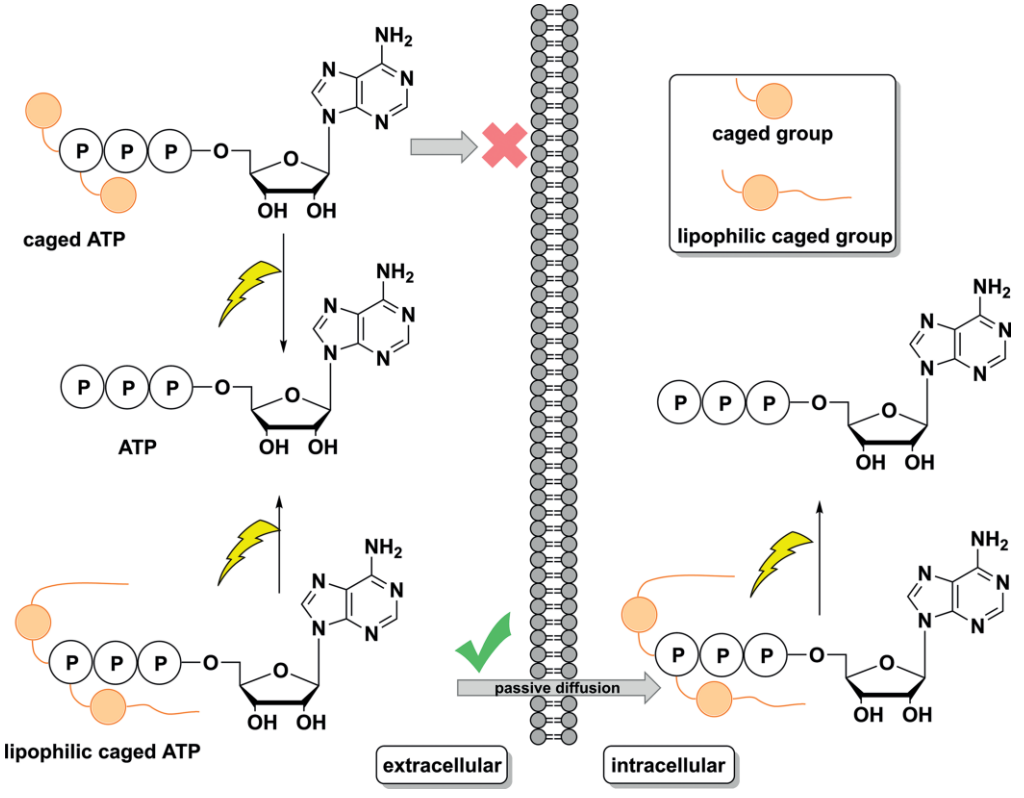


Scheme 1. Application of different photocaged ATP derivatives as potential extra- and intracellular precursors for ATP release.

(bathochromic shift) to avoid potential cell damage due to usage of energy-intense UV-B or UV-C light.[20,21] With these photocleavable groups we wanted to study in the future which protecting group is the most suitable for the application in extra- and intracellular context by irradiation with 365 nm UV-light. Furthermore, we also planned to synthesize derivatives of such donor substituted NB-masks which are derived from acetophenone starting materials. With these photomasks, the formation of a reactive and most likely toxic 2-nitrosobenzaldehyde by-product should be avoided due to the formation of a less reactive ketone.[20] Moreover, we modified the photolabile masks with lipophilic alkyl ether groups to potentially enable passive diffusion through cell membranes (Scheme 1). With these novel compounds not only extracellular but also intracellular processes may be investigated in the future after light-induced cleavage. To improve the lipophilicity and in contrast to the work of Kaplan and co-workers our novel compounds carry two photocleavable masks at ATPs γ-phosphate group instead of only one, relating to our extensive experience and reliable protocols for the synthesis of γ-bis-protected nucleoside triphosphates.[22,23]

For this reason and to preserve the comparability between the individual prepared derivatives, all synthesized compounds comprised two masks.

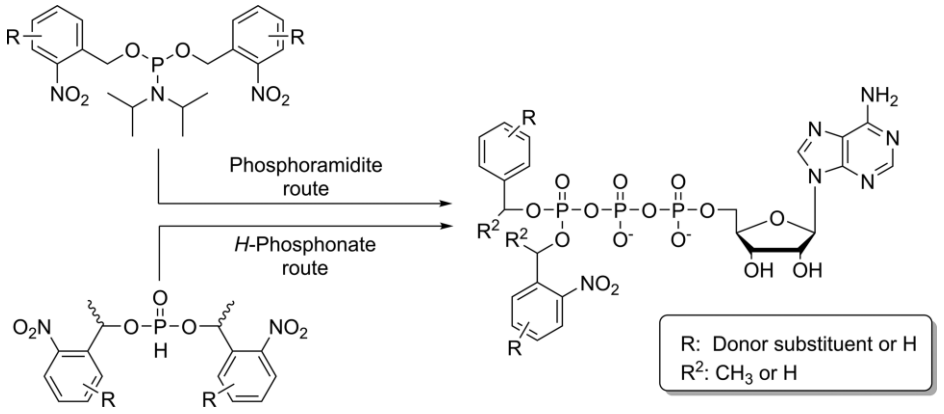
Finally, we demonstrated the versatility of our synthetic protocol in preparing mixed-type lipophilic photocaged-ATP by combining a simple photolabile group with an acyloxybenzyl (AB) mask known from our prodrug technology that, independently from the photocage, enhances the compound's lipophilicity. With this new type of compounds, it should be possible to generate a lock-in effect inside the cell, since the AB-group should be cleaved rapidly by esterase's after cell uptake and the formed monoprotected MeNV-ATP does not efflux from the cells again. Furthermore, the formed MeNV-ATP should be biologically inactive until the remaining photocaging group is cleaved by light and ATP gets released.

Results and Discussion

Chemical Synthesis of Novel Caged ATP Compounds

As we have demonstrated earlier, γ-modified nucleoside triphosphates can be synthesized in a three-step protocol, starting from a nucleoside monophosphate and an appropriately masked *H*-phosphonate.[24]

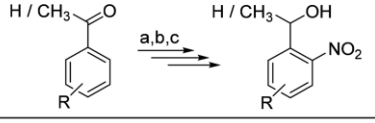
Following this protocol, the *H*-phosphonate was first activated by oxidative chlorination and subsequently reacted with inorganic phosphate to yield a non-symmetric pyrophosphate. Next, after stepwise activation this pyro-phosphate derivative was coupled with a nucleoside monophosphate, yielding the γ-masked nucleoside triphosphate. In a different approach to obtain β- or γ-masked nucleoside di- or triphosphates, we also reported on a highly reliable protocol using phosphoramidite chemistry.[25,26] In the present work, we transferred both strategies to the synthesis of γ-bis-caged nucleoside triphosphates by coupling of adenosine diphosphate with an appropriately masked phosphoramidite or *H*-phosphonate (Scheme 2).

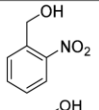
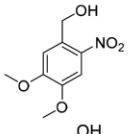
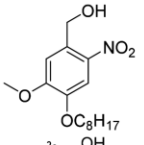
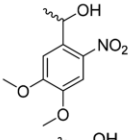
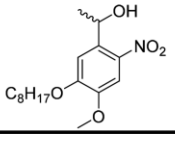


Scheme 2. Synthetic pathways for the preparation of γ-bis-photocaged ATP via phosphoramidite or *H*-phosphonate chemistry.

First, the different 2-nitrobenzyl derivatives **1–5** serving as synthetic precursors for the photocleavable units were synthesized starting from commercially available benzaldehyde and acetophenone derivatives. Table 1 summarizes the syntheses of these different photocleavable groups based on the 2-nitrobenzyl moiety.

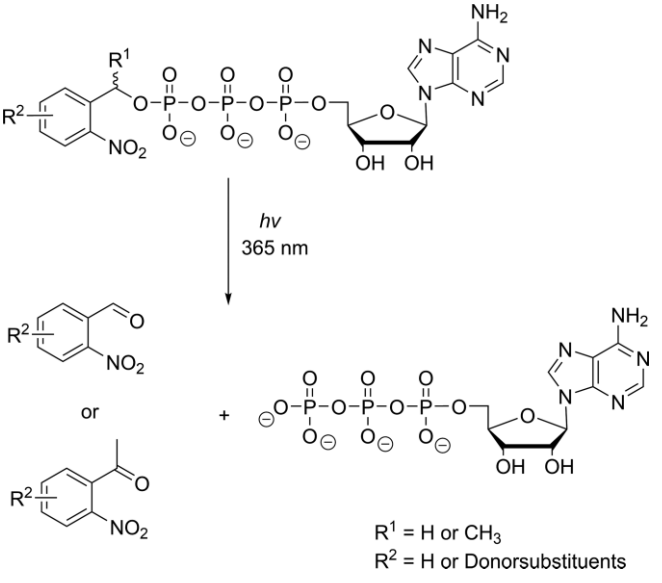
Table 1. Syntheses of different substituted photocleavable masks containing the *ortho*-nitro moiety (NB: nitrobenzyl, NV: nitroveratryl, *lip*: lipophilic).



Entry	R	Steps	Compound #	Product
1	-	c	**1** (NB)	
2	2-NO_2 3,4-OMe	c	**2** (NV)	
3	3-OH 4-OMe	a,b[1],c	**3** (*lip*NV)	
4	3,4-OMe	b[2],c	**4** (MeNV)	
5	3-OMe 4-OH	a,b[1],c	**5** (*lip*MeNV)	

[a] 1.2 equiv. alkyl halide, 3.0 equiv. K_2CO_3, DMF, 50 °C, overnight. [b[1]] HNO_3 (65 %), r.t., 48 h. [b[2]] HNO_3 (65 %), Ac_2O, 0 °C, 2 h. [c] 1–5–3.0 equiv. $NaBH_4$, MeOH/THF (1:1), 0 °C, 0.5–2 h.

The light-induced cleavage mechanism of these photoremovable groups based on the 2-nitrobenzyl moiety is well known and has been thoroughly investigated in the past.[27] By this mechanism, the 2-nitrobenzyl moiety undergoes a variety of transformations and in a final decaging step the desired substrate is released. Simultaneously the final step also provides a reactive nitroso aldehyde or less reactive nitroso ketone, depending on the used photocleavable group (Scheme 3).



Scheme 3. Photoinduced cleavage of NB-protected ATP. Depending on the used NB-derivative a reactive aldehyde or a less reactive ketone is formed.

The reactive and presumably toxic 2-nitrosobenzaldehyde by-product from photo-decaging of groups **1–3** (Table 1, Entry 1–3) can be avoided by introduction of a methyl group at the benzyl position (Table 1, Entry 4–5). Thus, a less reactive ketone is formed after photolysis. Furthermore, the cleavage rate of those secondary photocleavable groups is known to be faster than the cleavage of primary 2-nitrobenzyl derivatives.[20] A major disadvantage of these methyl groups is that they introduce a new stereogenic center into the target molecule. This led to a complex mixture of diastereomers. While this complex mixture can be avoided by an enantioselective reduction, the configuration of this stereogenic center has no known influence regarding the mechanism or rate of the photocleavage reaction. For that reason, so far, we did not perform a stereoselective synthesis or separation of the diastereomers but did our synthe-

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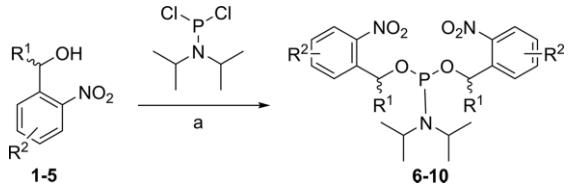
Table 2. Syntheses of symmetric phosphoramidites **6–10** starting from the 2-nitrobenzyl derivatives **1–5**.



Entry	2-Nitro-benzyl- derivative	R^1	R^2	Amidite	Yield [%]
1	**1**	H	–	**6**	83
2	**2**	H	3,4-OMe	**7**	92
3	**3**	H	3-OC_8H_{17}, 4-OMe	**8**	92
4	**4**	CH_3	3,4-OMe	**9**	–
5	**5**	CH_3	3-OMe, 4-OC_8H_{17}	**10**	–

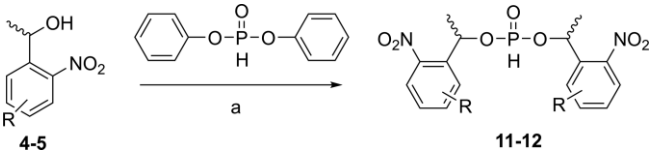
[a] 1.0 equiv. dichloro-*N*,*N*-diisopropylaminphosphoramidite, 2.2 equiv. 2-nitrobenzyl alcohol **1–6**, 2.5 equiv. Et_3N, THF, 0 °C, 18 h.

ses with the stereomeric mixture, respectively. Additionally, to increase the lipophilicity of the photocaged-ATP derivatives, two lipophilic masks were synthesized (**3**, **5**), both containing an octyl ether instead of the methoxy group (compare compounds **2** and **4,** Table 1). This was achieved by etherification of vanillin (Table 1, Entry 3) or 3-hydroxy-4-methoxyacetophenone (Table 1, Entry 5), respectively, with 1-bromooctane under alkaline conditions followed by nitration and finally, reduction of the carbonyl group. In each case, the product was obtained in nearly quantitative yields for both the etherification and reduction. The limiting step in both reaction procedures was the nitration, which was achieved by stirring the vanillin derivative for two days in concentrated nitric acid and the ketone derivative for two hours in a mixture of concentrated nitric acid and acetic anhydride, respectively. In both nitration reactions, an *ipso* substitution of the carbonyl group with the nitronium ion was observed, leading to the double nitrated side-product. The desired nitrated regioisomers **26** and **29** were obtained in approximately 60 % yield in both cases after purification by flash chromatography. Other nitration methods such as the standard method using a mixture of nitric and sulfuric acid led to complete decomposition of the starting materials, or in the case of dilute nitric acid, to no observable conversion at all. Importantly, depending on the commercial availability of the starting materials, not all these synthetic steps were necessary (Table 1). Next, we prepared phosphoramidites **6–10** as precursors for coupling reactions towards the photocaged-ATPs. As summarized in Table 2, phosphoramidites **6–8** were synthesized in excellent yields. However, the synthesis of phosphoramidites containing the methyl-substituted photocleavable groups **4** and **5** following the same protocol failed (Table 2, Entries 4 and 5). In comparison with starting materials **1–3** the more sterically demanding secondary benzyl alcohol in compounds **4** and **5** in combination with the rather bulky *N*,*N*-diisopropylamino moiety may have prevented the formation of phosphoramidites **9** and **10**.

To avoid the potential sterically hindrance during the phosphoramidite synthesis, we prepared symmetric *H*-phosphonates from these methyl-substituted compounds as potential precursors for the later coupling to photocleavable ATP.

In contrast to the phosphoramidites, *H*-phosphonates have no further bulky substituent at the phosphorus atom, and thus a steric interaction of the substituents is avoided. The corresponding *H*-phosphonates were obtained in yields of 33 % (**11**) and 72 % (**12**) by the reaction of diphenylphosphite with the secondary alcohols in small excess, as shown in Table 3. Starting from diphenylphosphite (DPP), phenolate is replaced by the photo-removable group **4** or **5**, most probably following an addition-elimination type mechanism. The moderate yields may be explained by the potential similar leaving group tendencies of the secondary alcohol and the phenol, hence resulting in a possible reverse reaction. With phosphoramidites **6–8** or the *H*-phosphonate **11–12** in hand, photocleavable ATP derivatives **13–17** were prepared by activation with dicyanoimidazole or *N*-chlorosuccinimide of those precursors followed by coupling with ADP in moderate to good yields as shown in Table 4.

Table 3. Synthesis of *H*-phosphonates **11–12** starting from the related derivatives **4–5**.



Entry	Secondary alcohol	R	Product	Yield [%]
1	**4**	3,4-OMe	**11**	33
2	**5**	3-OMe, 4-OC_8H_{17}	**12**	72

[a] 1.0 equiv. diphenylphosphite, 2.2 equiv. secondary alcohol derivative **11–12**, pyridine, r.t., 3 h.

Depending on the precursor, derivatives **13–17** were either synthesized by a 3,4-dicyanoimidazol-mediated coupling of phosphoramidites **6–8** with ADP and subsequent oxidation with *tert*-butylhydroperoxide (Table 4, Entry 1–3) or by oxidative chlorination of *H*-phosphonates **11–12** with *N*-chlorosuccinimide and subsequent coupling with ADP (Table 4, Entry 4–5). All photocaged-ATP derivatives were obtained in yields between 23–72 %. Comparing the different coupling strategies, it seems that the phosphoramidite approach led to the photocaged ATP in higher yields than the *H*-phosphonate approach. However, the difference in R^1 (H vs. CH_3) may also have played a role in this context. In terms of R^2, compounds **15** and **17** comprising the lipophilic octyl ether (Table 4, Entry 3, 5) were obtained in

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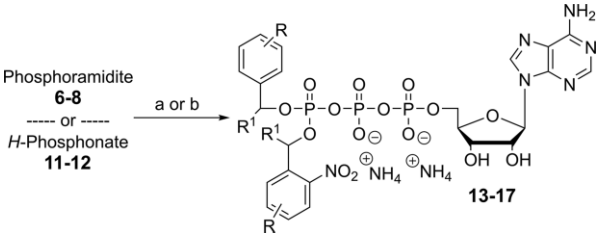
Table 4. Syntheses of photocleavable ATP derivatives **13–17**.



Entry	Starting material	Route	R^1	R^2	Product	Yield [%]
1	**6**	a	H	–	**13**	72
2	**7**	a	H	3,4-OMe	**14**	60
3	**8**	a	H	3-OC_8H_{17}, 4-OMe	**15**	38
4	**11**	b	CH_3	3,4-OMe	**16**	49
5	**12**	b	CH_3	3-OMe, 4-OC_8H_{17}	**17**	23

[a] 1.0 equiv. bis(tetrabutylammonium)-ADP, 1.5 equiv. amidite **6–8**, 1.5 eq DCI sol. (0.25 M in CH_3CN), 1 h, r.t., then 1.5 equiv. *t*BuOOH sol. (5.5 M in *n*-decane), CH_3CN, 20 min, r.t. [b] 1.0 equiv. *H*-phosphonate **11–12**, 2.0 equiv. NCS, 1 h r.t. then 0.8 equiv. bis(tetrabutylammonium)-ADP, CH_3CN, 1 h, r.t.

lower yields compared to the less lipophilic derivatives **13**, **14** and **16**. This observation can be explained by the lipophilic octyl moiety that caused a negative impact on the required solubility during the synthesis and also complicated the purification by automated reverse phase chromatography. We assumed that the increased amphiphilic properties of compounds **15** and **17** led to the formation of more complex structures in aqueous media (e.g. micelles) thus resulting in a non-trivial chromatographic purification of the compounds. Nonetheless, all caged ATP derivatives **13–17** were successfully prepared via the phosphoramidite or *H*-phosphonate route, and they were next evaluated regarding the release of ATP by photolysis.

Photolytic Release of ATP from Caged ATP Derivatives

For these experiments, solutions of 1 μmol/mL were prepared and irradiated with three 1300 mW UV-LEDs (365 nm, for more details, see experimental section). After defined intervals, aliquots were analyzed via HPLC to determine the progress of ATP formation (Figure 1). Based on the integral of the peaks assigned to starting material or ATP, half-lives and doubling times were calculated respectively for each caged-derivative and the released ATP **13–17** and the released ATP (Figure 2).

The photolytic cleavage was found to proceed stepwise for all caged-ATP derivatives. First, the formation of an intermediate with just one photocleavable mask was observed. During further irradiation, this intermediate subsequently was cleaved and released ATP. By comparison of the determined peak areas during the photolysis of compound **17**, it has to be considered, that the determined start value of the peak area for compound **17** is higher than the determined end value for the formed ATP since two UV-active groups are cleaved, thus lowering the cumulated UV absorption at 254 nm (Figure 2, lower part). Furthermore, the cleavage rate of the starting material and the intermediate decreased during the photolysis and simultaneously the formation of ATP became slower (Figure 1, lower part). This fact can also be observed by comparison of the calculated half-life and doubling times since all donor substituted caged-ATP derivatives showed a doubling time *k* more than twice as

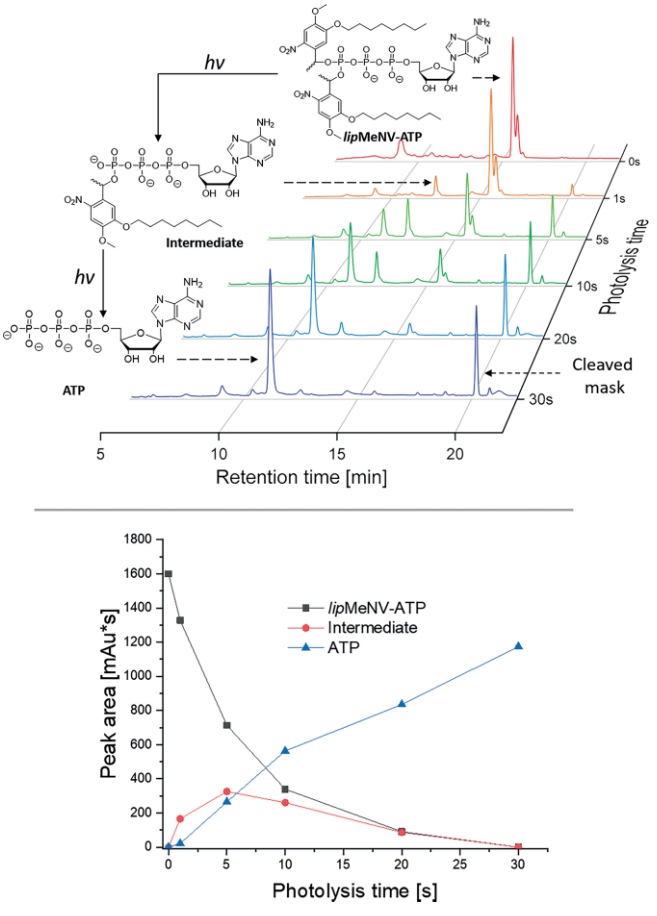


Figure 1. Upper part: representative HPLC chromatograms showing the time-course of the photolysis of lipMeNV-ATP (**17**) in PBS/CH_3CN, 1:1 (pH 7.4, r.t., 365 nm, 3 × 1300 mW). Due to the four different diastereomers of **17** that are not fully resolved by this HPLC-method, the starting material appears as a set of two peaks of different intensity. The main signals are labeled with the corresponding compounds. Lower part: determined peak areas plotted against photolysis time.

high than the calculated half-life. We assume that this observation can be explained by the fact that the released nitroso by-product absorbed the irradiated photons and thus the quan-

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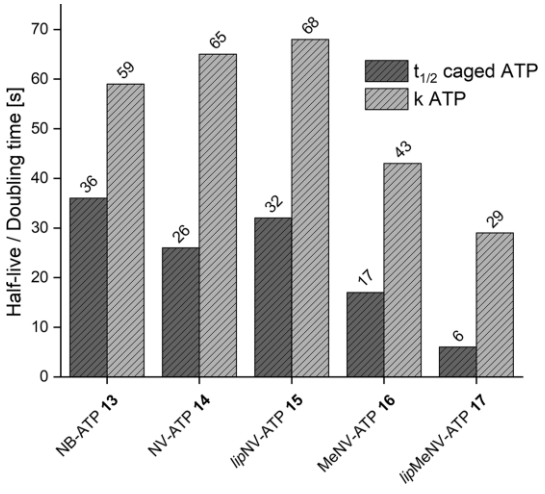


Figure 2. Calculated half-lives ($t_{1/2}$) and doubling times (k) for photolysis of caged-ATP derivatives and corresponding release of ATP for each derivative, respectively.

tum yield for the desired reaction is reduced. Only the non-substituted NB-ATP **13** did not show this trend (Figure 2).

The observation that NB-groups are cleaved almost as quickly as NV-groups by irradiation with 365 nm, despite poor absorption at this wavelength, was described in the literature.[28] A possible explanation could be the equimolar release of the respective nitroso by-product. During the photolysis of the lipophilic MeNV-ATP derivative **17**, we were able to observe the formation of the respective by-product via HPLC monitoring (Figure 1). As shown in Figure 3 the nitroso by-product has a strong absorption at 365 nm, thus reducing the available photons for the desired cleavage reaction.

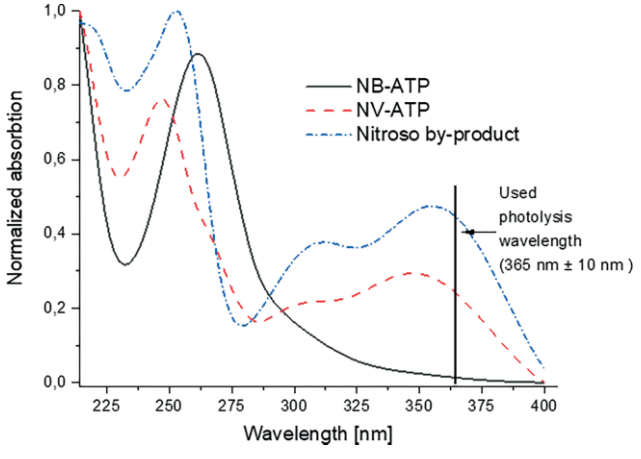


Figure 3. Measured UV/Vis spectra from the different non-lipophilic caged-ATP derivatives (nitroso by-product: 2-nitroso-4-methoxy-5-octanoxyacetophenon).

Since NB-ATP **13** did not show any relevant absorption at 365 nm, we assume that the corresponding nitroso by-product also showed only a low absorption at 365 nm, whereby the quantum yield is not affected.

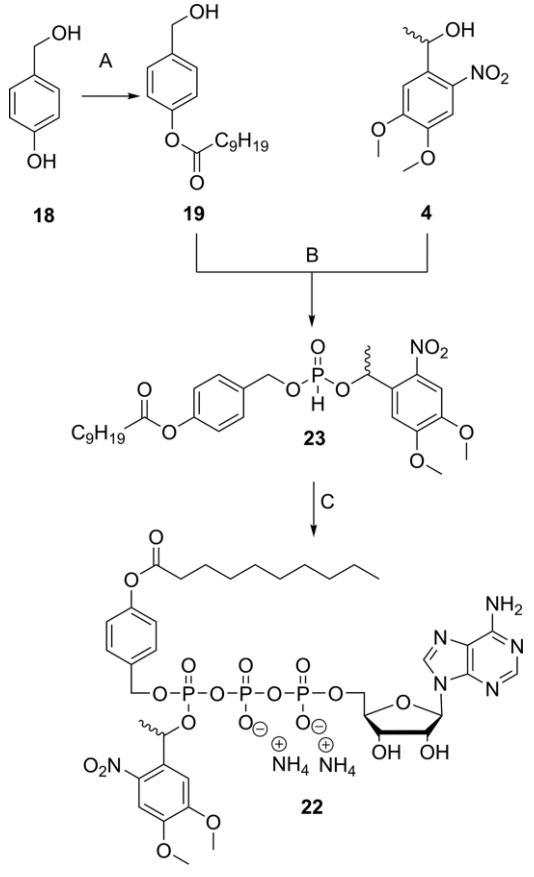
In line with exceptions a more rapid cleavage of the methyl-substituted MeNV ATP derivative **16** compared to the NV-protected ATP **14** was observed. This rapid cleavage is even more noteworthy by direct comparison of the lipophilic NV and MeNV derivatives **13** and **15**. The fact that the methyl substituent in the benzyl position led to a faster cleavage compared to their non-methylated derivatives is known in the literature.[19] Importantly, from the work presented here, it becomes apparent that the lipophilic modification in compound **17** almost triples the cleavage rate compared to the MeNV derivative **16**. This rapid cleavage of the lipophilic MeNV photo group is a promising feature opening up a new class of photolabile and lipophilic masked nucleoside triphosphates. However, a potential disadvantage for the intracellular application of these lipophilic compounds is still the cleaved by-product. During the photolysis reactions of lipophilic photocaged ATP **13** and **15**, the released by-product precipitated from the aqueous buffer, which may lead to cytotoxicity or interfere with the assay readout by potential intracellular applications.

In addition, to study whether the release of ATP also occurred by irradiation with visible light, the photolysis of the NB and NV derivative **13** and **14** was repeated at 405 nm, since it was reported that the NB-group can also be cleaved at 400 nm.[29] By usage of a 405 nm UV-light, with the exact power output like the previously used 365 nm UV-source, a markedly slower stepwise release of ATP, with half-lives of 102 s (NB) and 106 s (NV) for the ATP-derivatives **13** and **14** was observed. Thus, although it is possible to cleave the NB and NV groups with visible light, we would recommend the usage of 365 nm UV-light for a rapid release of ATP, since the release is three to four times faster than by usage of 405 nm light source. However, to avoid any potential cellular damages, it might be suitable to use visible light with the price of a slower release of ATP than with UV-light.

Combining Two Different γ-Masking Groups to Decouple Photocleavage and Lipophilicity

To overcome this problem, we synthesized a next-generation photocleavable and lipophilic ATP derivative, which gained its lipophilicity from a bio-reversible acyloxybenzyl group (AB-group) known from our Tri*PPP*ro-compounds, while a simple photocleavable group was present to exhibit all beneficial features of spatio-temporally controlled ATP-release. Since in the previous photolysis experiments the MeNV group had proven to be favorable over the other examined photocleavable masks, derivative **22** was chosen as prototypic target molecule. The synthesis of the asymmetric phosphoramidite precursor started from commercially available bis(*N*,*N*-diisopropylamino)-chlorophosphine which was first reacted with the MeNV mask **4.** Unexpectedly and in contrast to what we had observed for the symmetrical MeNV phosphoramidite **9**, we experienced no difficulties here and obtained phosphordiamidite **20** in excellent yields up to 90 %. As one possible explanation, the chloro substituent may be replaced more easily in this starting material. While, in the case of amidite **9**, the first MeNV substituent can interfere with the replacement of the second chloro substituent, here only one MeNV group was introduced and due to its free rotation around the P–O-C-bond, it may be possible that an already present MeNV group more strongly restricts the second

substitution reaction. The mixed phosphoramidite **21** was obtained by DCI-mediated coupling of diamidite **20** with 4-(hydroxymethyl)phenyl decanoate **19**, in almost quantitative yield. The lipophilicity providing AB-mask **19** was synthesized prior to esterification of the commercially available 4-hydroxybenzyl alcohol **18** with decanoyl chloride. The last step towards the desired mixed AB-MeVN-ATP **22** was the DCI-mediated coupling with ADP. Unfortunately, we were unable to isolate the ATP derivative **22** after repeated purification steps. For this reason, we again applied the *H*-phosphonate approach (Scheme 4), which had proven favorable for the synthesis of the symmetric MeNV protected derivatives **16** and **17**.



Scheme 4. Synthetic procedure for the preparation of AB-MeNV-ATP **22** via the *H*-phosphonate approach. **[A]:** 1.1 equiv. 4-hydroxybenzyl alcohol **18**, 1.0 equiv. decanoyl chloride, 1.0 equiv. Et_3N, THF, 20 min 0 °C, then 2 h r.t., 50 %; **[B]:** 1.0 equiv. DPP, 1.0 equiv. compound **4**, 1.0 equiv. compound **19**, pyridine, 45 min –15 °C then 2 h r.t., 38 %; **[C]:** 1.0 equiv. *H*-phosphonate **23**, 2.0 equiv. NCS, 1 h r.t. then 0.8 equiv. bis(tetrabutylammonium)-ADP, CH_3CN 1 h, r.t., 26 %.

The corresponding mixed protected *H*-phosphonate **23** was obtained starting from DPP after stepwise substitution of the phenolate groups by first the MeNV-group **4** and next the AB-group **19** in moderate yield. The coupling to the desired ATP derivative **22** was achieved with 26 % yield, which is in the same range as the lipophilic target compounds **17** (Table 4). After the successful synthesis of this mixed γ-phosphate protected ATP derivative **22**, the cleavage of each masking group was examined independently. To test the bioreversibility of the AB-mask, AB-MeNV-ATP **22** was incubated with pig-liver-esterase (PLE) in phosphate buffer (12 mM phosphate concentration, pH 7.4) at 37 °C. Aliquots were sampled at different time-points and analyzed by RP-HPLC equipped with a diode array detector. In an independent second experiment, AB-MeNV-ATP **22** was subjected to photolysis following the protocol of the previous experiments (Figure 1). The first experiment clearly showed that the bioreversible AB-group was cleaved rapidly, but the photocleavable MeNV group was stable under this simulated intracellular, esterase-containing conditions. The second experiment demonstrated, as expected, a rapid cleavage of the MeNV group by irradiation with UV-light, while the AB-group was stable under these conditions (Figure 4) The product in each experiment was identified based on the corresponding UV/Vis-spectra and retention times. After incubation of AB-MeNV-ATP **22** with esterase (PLE), the HPLC trace indicated the formation of a less lipophilic compound, while the observed peak area for the starting material was decreasing. In addition to the shift in

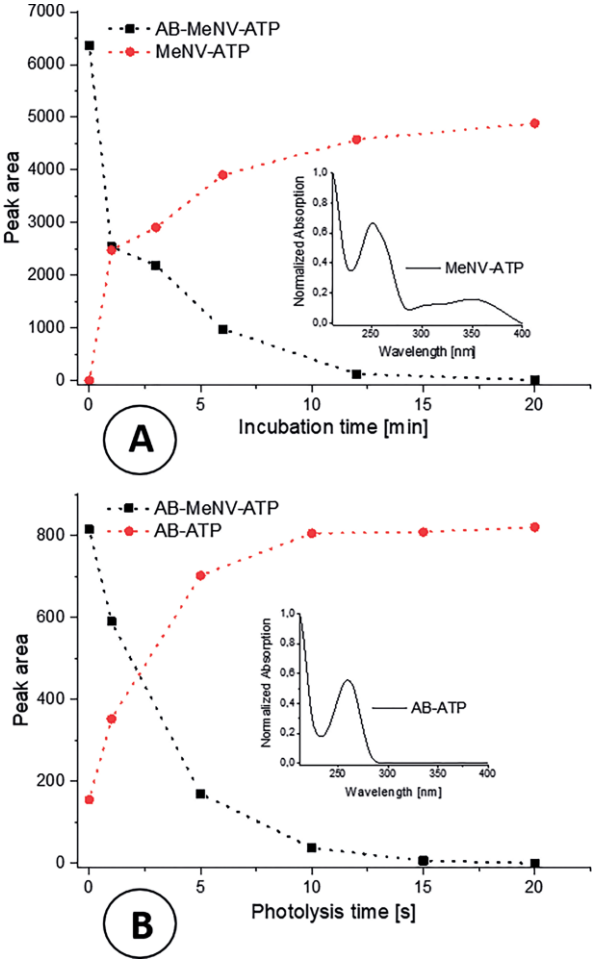


Figure 4. Panel A – Time-course of release of MeNV-ATP from AB-MeNV-ATP **22** via esterase-triggered hydrolysis (PLE). Conditions: PLE concentration: 100 U/mL, c(AB-MeNV-ATP) = 6 mM, 37 °C, pH 7.4; Panel B – Time-course of release of AB-ATP from AB-MeNV-ATP **22** during photolysis. Conditions: 3 × 1.3 W UV-LED, 365 nm (± 10 nm), 1 µmol/mL, r.t.; Peak areas were determined for panel A and B from measured RP-HPLC chromatograms (diode array detector, 254 nm). Inside each graph, the UV-spectrum of the corresponding formed product is shown. The presence of an absorption band at >300 nm indicates that the observed compound still includes the MeNV-masking unit.

the retention time of 2.1 minutes, the emerging signal still showed the typical absorption pattern of the MeNV group, indicating that

Indeed MeNV-ATP was formed (Figure 4 panel A). In contrast, the photolysis of the starting material **22** resulted in a minor shift in retention time (1.1 minutes), which indicated that only the MeNV was cleaved and that the AB-group was still covalently bound to ATP, providing higher lipophilicity and thus higher retention time compared to MeNV-ATP. Furthermore, in contrast to the first experiment, here the UV/Vis spectra of the emerging signal lack the typical MeNV absorption band (300–400 nm), thus indicating the cleavage of the MeNV-moiety (Figure 4 panel B). Formation of fully de-masked ATP was excluded since, for both experiments, the retention time of the product signal does not match with the retention time of ATP, which was proved by co-injection. The half-lives for AB-MeNV-ATP **22** were determined assuming simple decay as a mathematical model for the received HPLC signals. For the esterase-triggered cleavage of the AB-group (PLE, Figure 4, panel A), a half-life of 1.1 minutes was calculated and for the photolytic de-caging reaction (panel B) the calculated half-life was 2.2 seconds. The latter indicated that the photolysis of the MeNV group in compound **22** occurred even faster than in the bis-caged-MeNV-ATP **14** ($t_{1/2}$ = 6.0 s). This difference in cleavage speed can be explained by multiple possible reasons. First, the AB mask showed no absorption >300 nm, which means that all irradiated photons can be used for the desired cleavage reaction. In contrast, during the photolysis of symmetrically protected bis-MeNV-ATP **16**, a competition between the cleavage of the di-MeNV and the formed intermediate took place, which may increase the observed half-life. Furthermore, only one equivalent of reactive and potential toxic nitroso-by-product was formed during the photolysis of the mixed protected ATP **22** in comparison to bis-MeNV-ATP **16**, which liberated two equivalents of this unwanted UV-absorbing by-product. This means, that in the photolysis of **22** a minor number of photons was absorbed, thus increasing the cleavage rate.

After successful cleavage of each mask in the previously described independent experiments, we also demonstrated in one single experiment that a stepwise cleavage of both masks is feasible, as this is needed for potential cellular assays. For that, we incubated AB-MeNV-ATP **22** first with PLE as described before and after complete cleavage of the AB-group we carried out the photolysis and observed the quantitative formation of ATP. Consequently, these studies demonstrated that this new type of lipophilic bio-reversible masked *and* photocaged nucleoside triphosphate has promising properties for intracellular applications. On the one hand, lipophilicity can be specifically modified by the bio-reversible AB mask, and the photocleavable MeNV group may allow a precise and rapid release of the nucleoside triphosphate after potential cell uptake.

Conclusion

We successfully applied phosphoramidite as well as *H*-phosphonate chemistry for the synthesis of different γ-bis-caged ATP derivatives based on 2-nitrobenzyl photocleavable groups. By including short or long alkyl ether moieties, target compounds exhibiting different lipophilicities were obtained. All photocleavable groups were readily cleaved by irradiation of the target compounds with 365 nm UV-light and released ATP quantitatively. Moreover, we prepared a mixed protected ATP-derivative as an example for a new class of lipophilic photocaged nucleoside triphosphates. In this new class of compound, photocaging and lipophilicity are no longer coupled as it included one photocleavable MeNV-group and one acyloxybenzyl group that provided lipophilicity, as known from our Tri*PPP*ro-compounds. Furthermore, we demonstrated that in this mixed-type masked ATP, both groups can be cleaved orthogonally, releasing the corresponding mono-masked ATP-derivatives. In contrast to the prior synthesized lipophilic caged-ATP derivatives no water-insoluble by-products are formed, and the amount of potential toxic by-products is reduced by half. These properties make the synthesized mixed protected ATP derivative a promising tool to study cellular ATP-dependent processes like inflammation. Due to the enhanced lipophilicity, the mixed protected ATP should be able to be passively uptaken by the cell so that the potential assays are not influenced by invasive procedures such as micro-injection.

Experimental Section

General: All reactions except nitrations and reductions, were performed in oven-dried glassware, under nitrogen atmosphere, and with anhydrous solvents. THF, CH_2Cl_2, CH_3CN, and pyridine were dried with a MBraun (MB SPS-800) Solvent System and stored over nitrogen and molecular sieves (3 or 4 Å). DMF was purchased from Acros Organics anhydrous over molecular sieves. Triethylamine was heated to reflux over calcium hydride for two days and afterward stored over molecular sieves under nitrogen atmosphere and in the dark. All starting materials and reagents were purchased from Sigma Aldrich, TCI, Acros, ABCR or Carbosynth and were used without further purification. Ultrapure Water was obtained from a Sartorius arium pro apparatus (Sartopore 0.2 μm, UV) and HPLC-grade CH_3CN, used for automated reversed-phase chromatography and HPLC analysis, was purchased from VWR or Honeywell. Column chromatography was carried out with technical grade solvents, which were distilled prior to use, and silica gel MN 60 M (0.04–0.063 mm) from Macherey-Nagel. Precoated thin-layer-sheets (ALUGRAM® Xtra SIL G/UV_{254}, Macherey-Nagel) were used for thin-layer-chromatography. Spots were visualized by staining with a mixture of vanillin in sulfuric acid, acetic acid, and methanol, where possible. P^{+III}-containing compounds were stained with an alkaline permanganate solution. Automated reversed-phase flash chromatography was performed using prepacked MN RS 40 C_{18} ec columns on an Interchim Puriflash 430 or a Büchi Sepacore system. For all automated reversed-phase chromatography, a gradient from 100 % ultrapure water to 100 % CH_3CN within 20 min was used (flowrate: 20 mL/min). All NMR solvents were purchased from Euriso-Top or Deutero. NMR spectra were recorded on Bruker instruments Fourier HD 300, Avance I 400, DRX 500, or Avance III 600 at room temperature. All proton and carbon NMR spectra were calibrated by the respective solvent signal. High-resolution mass spectra were measured with an Agilent 6224 ESI-TOF instrument und IR spectra were obtained on a Bruker Alpha IR spectrometer.

HPLC: Chromatograms were recorded on an Agilent 1260 Infinity II with the following components: G7129A Vialsampler, G7111A Quat

Pump, G7116A MCT, G7117C DAD HS. All adenosine-containing signals were detected at 254 nm with the following method: 0–25 min 5 % CH_3CN in 2 mM aq. tetrabutylammonium acetate buffer (pH 6.0) 5 % to 100 % CH_3CN, 1mL/min.

Photolysis Studies: A solution of the test compound at 1 mg/mL was dissolved in a 1:1-mixture of PBS (pH 7.4) and CH_3CN in a small glass vial (diameter: 11 mm). The solution was stirred with a small stirring rod on a magnetic stirrer and the LED-UV source was placed directly in front of the vial. The LEDs were arranged in a triangle on a passive cooling apparatus (3 × 1400mW, NVSU233A-U365, Sahlmann Photochemical Solutions or 3 × 1400mW, NVSU233A-405, Sahlmann Photochemical Solutions). After fixed intervals, aliquots of 20 μL were analyzed via HPLC (injection volume: 7 μL; method: see HPLC-section).

PLE Hydrolysis: To a mixture of 60 μL DMSO and 400 μL PBS (pH 7.4) 40 μL of a 50 mM stock solution of the related caged-ATP derivative in DMSO was added. To this solution, 30 μL PLE solution (100 U/mL) was added. Yielding a 6 mM solution of the caged-ATP-derivative and a final volume of 530 μL. The mixture was incubated at 37 °C and at fixed intervals, aliquots of 35 μL were analyzed via HPLC (injection volume 30 μL, method: see HPLC-section).

Synthesis

General Procedure 1. Reduction of Carbonyl Groups: The respective aldehyde or ketone was dissolved in a 1:1 mixture of methanol and THF under ambient atmosphere and cooled to 0 °C (amount of volume listed in the appropriate synthesis protocol). To the resulting solution sodium borohydride (1.5–3.0 equiv.) was added in portions and the suspension was stirred for 1–3 hours at room temperature. All volatile substances were removed in vacuo and the remaining residue was taken up in water and ethyl acetate. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried with sodium sulfate, and concentrated in vacuo. After removal of the solvent, the desired alcohol was obtained in high purity without further purification.

General Procedure 2: Synthesis of Phosphoramidites: Under nitrogen atmosphere (diisopropylamino)dichlorophosphine was dissolved in 5 mL anhydrous THF and added dropwise to an ice-cold solution of the corresponding alcohol (2.1 equiv.) and dry triethylamine (3.0 equiv.) in 15 mL THF. The solution was stirred at room temperature for 18 h in the dark. The precipitated solid was removed by filtration and the filtrate was concentrated to dryness in vacuo. Finally, the crude product was purified by column chromatography on silica gel (PE/EtOAc, 10:1 or 1:1 + 5 % TEA).

General Procedure 3: Synthesis of *H*-phosphonates: Under nitrogen atmosphere diphenylphosphite was dissolved in anhydrous pyridine. Next, the corresponding alcohol was added and the solution was stirred for 2–3 h at room temperature. All volatile compounds were removed in vacuo and the residue was co-evaporated several times with toluene and CH_2Cl_2. Finally, the crude product was purified by column chromatography on silica gel (PE/EtOAc, 2:1 to pure EtOAc).

General Procedure 4: Synthesis of Caged-ATP via Phosphoramidites: The corresponding phosphoramidite (1.5 equiv.) and ADP (as 2.0–2.5 tetrabutylammonium salt) were combined as solids, dried in high-vacuo for three hours, and subsequently dissolved in anhydrous CH_3CN. To the stirred solution, DCI-activator (0.25 M in CH_3CN) was added portion-wise (1 × 0.5 equiv., 4 × 0.25 equiv.) at room temperature. Between each portion, the solution was stirred for five minutes. After complete addition, the reaction was stirred for another 30 minutes at room temperature. Afterward, *t*BuOOH (5.5 M in *n*-decane, 1.5 equiv.) was added and the solution was stirred again for 30 minutes. All volatile components were removed in vacuo. The crude product was purified by automated RP flash column chromatography. Fractions were pooled and the solvent was removed by lyophilization. The residue was dissolved in a minimum of water and cations were exchanged to ammonium using DOWEX (50WX8, 50–100 mesh), followed by a second automated RP flash column chromatography.

General Procedure 5: Synthesis of Caged-ATP via *H*-Phosphonates: The corresponding *H*-phosphonate (1.0 equiv.) was dried in high vacuo for one hour and subsequently dissolved in anhydrous CH_3CN. To the resulting solution, *N*-chlorosuccinimide (2.0 equiv.) was added as a solid and the solution was stirred for one hour at room temperature. Then, ADP (2.0–2.5 × tetrabutylammonium salt, 0.8 equiv.) was added and the solution was stirred for one hour at room temperature. All volatile components were removed in vacuo. The crude product was purified by automated RP flash column chromatography. Fractions were pooled and the solvent was removed by lyophilization. The residue was dissolved in a minimum of water and cations were exchanged to ammonium using DOWEX (50WX8, 50–100 mesh), followed by a second automated RP flash column chromatography.

Adenosine Diphosphate Tetrabutylammonium Salt (24): ADP (Na-salt, 0.82 g, 1.7 mmol, 1.0 equiv.) was dissolved in 5 mL water and transferred by ion-exchange chromatography on DOWEX (50WX8, 50–100 mesh) into the protonated species. 12.2 mL (10 % in water, 4.3 mmol, 2.5 equiv.) tetrabutylammonium hydroxide solution was added and the resulting solution was stirred for 30 minutes. The solvent was removed by lyophilization and the remaining residue was purified by automated RP flash column chromatography. The solvent was again removed by lyophilization and the residue was dried in high vacuo. Yield 1.66 g (1.7 mmol, quant.) as a pale yellow hygroscopic solid. ^{1}H-NMR (500 MHz, D_2O): δ = 8.53 (s, 1 H), 8.19 (s, 1 H), 6.09 (d, $^3J_{H,H}$= 5.8 Hz, 1 H), 4.75–4.70 (m, 1 H), 4.58 (dd, $^3J_{H,H}$ = 5.2 Hz, $^3J_{H,H}$ = 3.8 Hz, 1 H), 4.37–4.33 (m, 1 H), 4.28–4.20 (m, 1 H), 4.19–4.12 (m, 1 H), 3.22–3.04 (m, 16 H, CH_2), 1.67–1.52 (m, 16 H, CH_2), 1.39–1.24 (m, 16 H, CH_2), 0.90 (t, $^3J_{H,H}$ = 7.4 Hz, 24 H, CH_3); ^{13}C-NMR (125 MHz, D_2O): δ = 155.5, 152.7, 148.9, 139.9, 118.4, 86.8, 84.0 (d, $^3J_{C,P}$ = 9.1 Hz), 74.4, 70.1, 64.6 (d, $^2J_{C,P}$ = 5.4 Hz), 58.0 (CH_2), 23.0 (CH_2), 19.1 (CH_2), 12.8 (CH_3); ^{31}P-NMR (200 MHz, D_2O): δ = –8.23 (d, $^2J_{P,P}$ = 22.1 Hz), –11.2 (d, $^2J_{P,P}$ = 22.1 Hz).

(Diisopropylamino)dichlorophosphine (25): The synthesis of compound **25** was performed as described in the literature. The analytical data is in accordance with the literature.[30]

Bis(2-nitrobenzyl)-*N*,*N*-diisopropylphosphoramidite (6): The synthesis of compound **6** was performed as described in the literature. Purification was carried out by column chromatography with PE/EtOAc, 1:1 + 5 % TEA on silica gel. The analytical data is in accordance with the literature.[31]

Bis(4,5-dimethoxy-2-nitrobenzyl)-*N*,*N*-diisopropylphosphoramidite (7): The synthesis of compound **7** was performed as described in the literature. Purification was carried out by column chromatography with PE/EtOAc, 1:1 + 5 % TEA on silica gel. The analytical data is in accordance with the literature.[31]

3-Methoxy-4-octanoxybenzaldehyde (26): Under anhydrous conditions 3.00 g (19.7 mmol, 1.0 equiv.) vanillin and 8.35 g (59.2 mmol, 3.0 equiv.) freshly dried potassium carbonate was suspended in 50 mL dry DMF. To the resulting suspension 5.2 mL (5.8 g, 30 mmol, 1.5 equiv.) *n*-bromooctane were added. The mixture was stirred at 50 °C for 18 h. After full conversion 50 mL 1 M NaOH-soln. was added and the mixture was extracted three times with diethyl ether. The combined organic layers were washed with brine, dried with

sodium sulfate, and concentrated in vacuo. The remaining residue was purified on silica gel (CH_2Cl_2/EtOAc, 1:1). Yield: 5.0 g (19 mmol, 96 %) as a colorless solid. R_f = 0.41 (DCM/EtOAc, 1:1); m.p. 35 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.84 (s, 1 H, C*H*O), 7.43 (dd, $^3J_{H,H}$ = 8.1 Hz, $^4J_{H,H}$ = 1.9 Hz, 1 H, H-Ar), 7.40 (d, $^4J_{H,H}$ = 1.9 Hz, 1 H, H-Ar), 6.96 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, H-Ar), 4.10 (t, $^3J_{H,H}$ = 6.9 Hz, 2 H, CH_2), 3.92 (s, 3 H, OMe), 1.88 (p, $^3J_{H,H}$ = 7.3 Hz, 2 H, CH_2), 1.50–1.43 (m, 2 H, CH_2), 1.40–1.28 (m, 8 H, 4 × CH_2), 0.88 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 191.7 (*C*HO), 154.4 (C-Ar), 150.0 (C-Ar), 130.0 (C-Ar), 127.0 (C-Ar), 111.5 (C-Ar), 109.4 (C-Ar), 69.3 (CH_2), 56.2 (OMe), 31.9, 29.4, 29.3, 22.8, 29.0, 26.0 (6 × CH_2), 14.2 (CH_3); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3338, 3080, 3005, 2964, 2766, 1681, 1669, 1583, 1507, 1464, 1405, 1390, 1266, 1197, 1132, 1065, 1011, 958, 790, 657, 571; HRMS (ESI$^+$): *m/z* calcd. for $C_{16}H_{24}O_3$ + H$^+$: 265.1798, found 265.1799.

5-Methoxy-2-nitro-4-octanoxybenzaldehyde (27): Under ambient conditions 45 mL conc. nitric acid (65 %) was cooled to 0 °C and 1.77 g (6.70 mmol, 1.0 equiv.) of aldehyde **26** were added in small portions. The resulting solution was stirred for two days at room temperature. The reaction mixture was slowly poured into 200 mL ice water. The mixture was extracted with ethyl acetate twice. The combined organic layers were washed with sat. sodium bicarbonate solution and brine. The organic layer was dried with sodium sulfate and concentrated in vacuo. The remaining residue was purified on silica gel using a gradient (PE/CH_2Cl_2, 1:1 to pure CH_2Cl_2). Yield: 1.19 g (3.85 mmol, 58 %) as a yellow solid. R_f = 0.30 (CH_2Cl_2); m.p. 67 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 10.43 (s, 1 H, C*H*O), 7.59 (s, 1 H, H-Ar), 7.41 (s, 1 H, H-Ar), 4.14 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, CH_2), 4.00 (s, 3 H, OMe), 1.90 (p, $^3J_{H,H}$ = 7.1 Hz, 2 H, CH_2), 1.51–1.45 (m, 2 H, CH_2), 1.40–1.25 (m, 8 H, 4 × CH_2), 0.88 (t, $^3J_{H,H}$ = 6.9 Hz, 3 H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 187.9 (*C*HO), 153.6 (C-Ar), 152.2 (C-Ar), 144.0 (C-Ar), 125.3 (C-Ar), 110.0 (C-Ar), 108.1 (C-Ar), 70.1 (CH_2), 56.8 (OMe), 31.9, 29.4, 29.3, 28.9, 26.0, 22.8 (6 × CH_2), 14.2 (CH_3); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3013, 2960, 2914, 2852, 1685, 1602, 1570, 1513, 1481, 1408, 1378, 1330, 1222, 1170, 1084, 1058, 1036, 1027, 983, 810, 756, 719, 607; HRMS (ESI$^+$): *m/z* calcd. for $C_{16}H_{23}NO_5$ + H$^+$: 310.1649, found 310.1648.

5-Methoxy-2-nitro-4-octanoxybenzyl Alcohol (3): The synthesis was done according to general procedure 1; 0.75 g (2.4 mmol, 1.0 equiv.) of aldehyde **27** was dissolved in 30 mL THF/MeOH, 1:1 and 0.14 g (3.6 mmol, 1.5 equiv.) sodium borohydride was added. Yield: 0.72 g (2.3 mmol, 96 %) as a yellow solid. R_f = 0.43 (CH_2Cl_2/MeOH, 19:1); m.p. 69 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.69 (s, 1 H, H-Ar), 7.14 (s, 1 H, H-Ar), 4.94 (s, 2 H, Ph-CH_2), 4.07 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, CH_2), 3.98 (s, 3 H, OMe), 2.65 (s, 1 H, OH), 1.87 (p, $^3J_{H,H}$ = 7.2 Hz, 2 H, CH_2), 1.50–1.44 (m, 2 H, CH_2), 1.39–1.25 (m, 8 H, 4 × CH_2), 0.88 (t, $^3J_{H,H}$ = 6.9 Hz, 3 H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 154.4 (C-Ar), 147.7 (C-Ar), 139.9 (C-Ar), 132.0 (C-Ar), 111.4 (C-Ar), 109.4 (C-Ar), 69.7 (CH_2), 63.1 (Ph-*C*H$_2$), 56.6 (OMe), 31.9, 29.4, 29.3, 29.0, 26.0 22.8 (6 × CH_2), 14.2 (CH_3); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3538, 3103, 3013, 2951, 2923, 2853, 1577, 1513, 1503, 1468, 1440, 1400, 1347, 1324, 1209, 1155, 1069, 1045, 975, 878, 810, 736, 716, 683, 506; HRMS (ESI$^+$): *m/z* calcd. for $C_{16}H_{25}NO_5^+$- H_2O: 294.1705, found 294.1378.

Bis(5-methoxy-2-nitro-4-octanoxybenzyl)-*N,N*-diisopropylphosphoramidite (8): The synthesis was done according to general procedure 2; 0.19 mL (0.92 mmol, 0.19 g 1.0 equiv.) of compound **25** was added to 0.60 g (1.9 mmol, 2.1 equiv.) of alcohol **3** and 0.38 mL (0.28 mmol, 0.28 g) triethylamine. Column chromatography was done with PE/EtOAc, 10:1 + 5 % TEA. Yield: 0.64 g (0.85 mmol, 92 %) as a yellow oil. R_f = 0.70 (PE/EtOAc + 5 % TEA, 10:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.72 (s, 2 H, H-Ar), 7.39 (s, 2 H, H-Ar), 5.24 (dd, $^3J_{H,P}$ = 6.8 Hz, $^2J_{H,H}$ = 16.3 Hz, 2 H, Ph-CH_2-a), 5.15 (dd, $^3J_{H,P}$ = 6.9 Hz, $^2J_{H,H}$ = 16.4 Hz, 2 H, Ph-CH_2-b), 4.06 (t, $^3J_{H,H}$ = 6.8 Hz, 4 H, CH_2), 3.94 (s, 6 H, OMe), 3.82–3.74 (m, 2 H, NC*H*), 1.86 (p, $^3J_{H,H}$ = 7.2 Hz, 4 H, CH_2), 1.50–1.44 (m, 4 H, CH_2), 1.39–1.25 (m, 16 H, 8 × CH_2), 1.27 (d, $^3J_{H,H}$ = 6.9 Hz, 12 H, CH_3), 0.88 (t, $^3J_{H,H}$ = 6.9 Hz, 6 H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 154.4 (C-Ar), 147.2 (C-Ar), 138.8 (C-Ar), 131.6 (d, $^3J_{C,P}$ = 8.1 Hz, C-Ar), 109.5 (C-Ar), 109.2 (C-Ar), 69.7 (CH_2), 62.7 (d, $^2J_{C,P}$ = 19.5 Hz, Ph-CH_2), 56.5 (OMe), 43.7 (d, $^2J_{C,P}$ = 12.2 Hz, N-*C*), 31.9, 29.4, 29.3, 29.0, 26.0, 22.8 (6 × CH_2), 24.9 (d, $^3J_{C,P}$ = 7.2 Hz, CH_3), 14.2 (CH_3); ^{31}P-NMR (162 MHz, $CDCl_3$): δ = 147.3; IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3106, 2961, 2925, 2855, 1576, 1516, 1462, 1432, 1399, 1370, 1323, 1125, 1020, 996, 976, 870, 818, 750, 724, 668; HRMS (ESI$^+$): *m/z* calcd. for $C_{38}H_{62}N_3O_{10}P$ + H$^+$: 752.4246, found 752.4240.

4,5-Dimethoxy-2-nitroacetophenone (28): Under ambient conditions 10.18 g (56.47 mmol, 1.0 equiv.) 3,4-dimethoxyacetophenone were dissolved in 30 mL acetic anhydride. The solution was slowly added to an ice-cold solution of 60 mL conc. nitric acid (65 %) and 1.5 mL acetic anhydride. After complete addition, the solution was stirred for one hour at room temperature. The solution was poured carefully into 300 mL ice-water. After filtration, the precipitate was washed with ice-cold water until the water was nearly colorless. The solid was recrystallized from a mixture of water and ethanol (1:1). Yield: 8.19 g (36.4 mmol, 64 %) as yellow needles. R_f = 0.45 (CH_2Cl_2); m.p. 134 °C; ^{1}H-NMR (400 MHz, [D$_6$]DMSO): δ = 7.64 (s, 1 H, H-Ar), 7.22 (s, 1 H, H-Ar), 3.92 (s, 3 H, OMe), 3.90 (s, 3 H, OMe), 2.52 (s, 3 H, CH_3); ^{13}C-NMR (100 MHz, [D$_6$]DMSO): δ = 199.3 (*C*=O), 153.2 (C-Ar), 149.4 (C-Ar), 138.4 (C-Ar), 131.1 (C-Ar), 109.7 (C-Ar), 107.1 (C-Ar), 56.6 (OMe), 56.3 (OMe), 30.0 (CH_3). IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2973, 2931, 2842, 1699, 1619, 1574, 1508, 1462, 1421, 1394, 1345, 1322, 1181, 1113, 1043, 1020, 953, 881, 788, 697, 666, 599, 536; HRMS (ESI$^+$): *m/z* calcd. for $C_{10}H_{11}NO_5$ + H$^+$: 226.0715, found 226.0707.

***rac*-1-(4,5-Dimethoxy-2-nitrophenyl)ethane-1-ol (4):** The synthesis was done according to general procedure 1; 0.95 g (4.2 mmol, 1.0 equiv.) of ketone **28** was dissolved in 30 mL THF/MeOH, 1:1 and 0.61 g (13 mmol, 3.0 equiv.) sodium borohydride was added. Yield: 0.94 g (4.1 mmol, 99 %) as a yellow solid. R_f = 0.12 (CH_2Cl_2); m.p. 123 °C; ^{1}H-NMR (400 MHz, [D$_6$]DMSO): 7.53 (s, 1 H, H-Ar), 7.36 (s, 1 H, H-Ar), 5.47 (d, $^3J_{H,H}$ = 4.7 Hz, 1 H, OH), 5.26 (dt, $^3J_{H,H}$ = 4.4 Hz, $^4J_{H,H}$ = 6.2 Hz, 1 H, Ar-C*H*-CH_3), 3.90 (s, 3 H, OMe), 3.84 (s, 3 H, OMe), 1.37 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3); ^{13}C-NMR (100 MHz, [D$_6$]DMSO): 153.2 (C-Ar), 147.1 (C-Ar), 138.9 (C-Ar), 137.9 (C-Ar), 108.9 (C-Ar), 107.3 (C-Ar), 63.9 (C-Ar-*C*H-CH_3), 56.0 (2 × OMe), 25.1 (CH_3); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3287, 3113, 2965, 2928, 2897, 1614, 1580, 1523, 1499, 1466, 1439, 1362, 1220, 1190, 1164, 1037, 1019, 970, 867, 796, 758, 659, 592. HRMS (ESI$^+$): *m/z* calcd. for $C_{10}H_{13}NO_5^+$- H_2O: 210.0766, found 210.0755.

Bis(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)phosphonate (11): The synthesis was done according to general procedure 3; 0.41 g (1.8 mmol, 2.1 equiv.) of alcohol **4** was dissolved in 10 mL pyridine and 0.17 mL (0.21 g, 0.90 mmol, 1.0 equiv.) diphenylphosphite was added. Purification was done by column chromatography: PE/EtOAc, 2:1 to EtOAc. Yield: 0.14 g (0.32 mmol, 31 %) as a yellow solid and mixture of four diastereomers. ^{1}H and ^{13}C-NMR data are provided for one pure isolated diastereomer. R_f = 0.55 (EtOAc); ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.46 (s, 1 H, H-Ar), 7.42 (s, 1 H, H-Ar), 7.04 (s, 1 H, H-Ar), 6.96 (d, $^1J_{H,P}$ = 703 Hz, 1 H, P-H), 6.94 (s, 1 H, H-Ar), 6.27 (dq, $^3J_{H,P}$ = 9.6 Hz, $^3J_{H,H}$ = 6.3 Hz, 1 H, Ph-C*H*-P), 6.13 (dq, $^3J_{H,P}$ = 10.3 Hz, $^3J_{H,H}$ = 6.3 Hz, 1 H, Ph-C*H*-P), 3.96–3.88 (m, 12 H, OMe), 1.66 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.62 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 154.1 (C-Ar), 153.9 (C-Ar), 148.3 (C-Ar), 148.3 (C-Ar), 138.7 (C-Ar), 138.6 (C-Ar), 132.4 ($^3J_{C,P}$ = 3.9 Hz, C-Ar), 132.2 ($^3J_{C,P}$ = 5.2 Hz, C-Ar), 107.5 (C-Ar), 107.5 (C-Ar), 108.1 (C-

Ar), 108.1 (C-Ar), 71.0 (d, $^2J_{C,P}$ = 4.0 Hz, Ar-*C*-P), 70.5 (d, $^2J_{C,P}$ = 5.0 Hz, Ar-*C*-P), 56.7,56.6, 56.5, 56.4 (OMe), 25.0 (d, $^3J_{C,P}$ = 3.4 Hz, CH_3), 24.7 (d, $^3J_{C,P}$ = 4.8 Hz,CH_3); ^{31}P-NMR (242 MHz, $CDCl_3$): δ = 5.1; IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2976, 2938, 2849, 1615, 1581, 1519, 1461, 1378, 1330, 1273, 1172, 1105, 1041, 1000, 951, 873, 647, 599; HRMS (ESI^+): *m/z* calcd. for $C_{20}H_{25}N_2O_{11}P + Na^+$: 523.1094, found 523.1053.

4-Methoxy-3-octanoxyacetophenone (29): Under anhydrous conditions 0.97 g (5.8 mmol, 1.0 equiv.) 3-hydroxy-4-methoxyacetophenone and 2.7 g (20 mmol, 3.5 equiv.) freshly dried potassium carbonate was suspended in 50 mL dry DMF. To the resulting suspension 2.7 mL (1.7 g, 30 mmol, 1.5 equiv.) *n*-bromooctane were added. The mixture was heated to 50 °C and stirred for 18 h. After full conversion 50 mL 1 M NaOH-sol. were added and the mixture was extracted three times with diethyl ether. The combined organic layers were washed with brine, dried with sodium sulfate, and concentrated in vacuo. The remaining residue was purified on silica gel (CH_2Cl_2/EtOAc, 1:1). Yield: 1.45 g (5.21 mmol, 90 %) as a colorless solid. R_f = 0.40 (CH_2Cl_2/EtOAc, 1:1); m.p. 46 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.55 (dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 2.0 Hz, 1 H, H-Ar), 7.51 (d, $^3J_{H,H}$ = 2.0 Hz, 1 H, H-Ar), 6.88 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, H-Ar), 4.06 (t, $^3J_{H,H}$ = 6.9 Hz, 2 H, CH_2), 3.93 (s, 3 H, OMe), 2.56 (s, 3 H, CH_3), 1.85 (p, $^3J_{H,H}$ = 7.2 Hz, 2 H, CH_2), 1.49–1.43 (m, 2 H, CH_2), 1.38–1.25 (m, 8 H, H-Alkyl), 0.88 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 197.0 (*C*=O), 153.8 (C-Ar), 148.7 (C-Ar), 130.6 (C-Ar), 123.2 (C-Ar), 111.7 (C-Ar), 110.3 (C-Ar), 69.2 (CH_2), 56.2 (OMe), 31.9, 29.5, 29.3, 29.2, 26.1, 22.8 (6 × CH_2), 26.4 (CH_3), 14.2 (CH_3); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2962, 2940, 2921, 2851, 1671, 1583, 1507, 1464, 1444, 1424, 1344, 1286, 1215, 1185, 1143, 1072, 1003, 977, 877, 723, 642, 591; HRMS (ESI^+): *m/z* calcd. for $C_{17}H_{26}O_3 + H^+$: 279.1950, found 279.1960.

4-Methoxy-2-nitro-5-octanoxyacetophenone (30): Under ambient conditions, 1.26 g (4.53 mmol, 1.0 equiv.) of ketone **29** were dissolved in 23 mL acetic anhydride. The solution was slowly added to an ice-cold solution of 30 mL conc. nitric acid (65 %) and 1.5 mL acetic anhydride. After complete addition, the solution was stirred for one hour at room temperature. The solution was poured carefully into 200 mL ice-water. The suspension was extracted twice with CH_2Cl_2 and the combined organic layers were washed with sat. sodium bicarbonate solution and brine, dried with sodium sulfate, and concentrated in vacuo. The remaining residue was purified by column chromatography on silica gel (CH_2Cl_2). Yield: 830 mg (2.57 mmol, 57 %) as a yellow solid. R_f = 0.47 (CH_2Cl_2); m.p. 42 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.60 (s, 1 H, H-Ar), 6.73 (s, 1 H, H-Ar), 4.09 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, CH_2), 3.96 (s, 3 H, OMe), 2.49 (s, 3 H, CH_3), 1.87 (p, $^3J_{H,H}$ = 7.1 Hz, 2 H, CH_2),1.49–1.42 (m, 2 H,CH_2), 1.39–1.25 (m, 8 H, 4 × CH_2), 0.88 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 200.3 (*C*=O), 153.9 (C-Ar), 150.0 (C-Ar), 138.3 (C-Ar), 133.0 (C-Ar), 109.6 (C-Ar), 107.1 (C-Ar), 70.0 (CH_2), 56.7 (OMe), 36.5 (CH_3), 31.9, 29.4, 29.3, 28.9, 25.9, 22.8 (6 × CH_2), 14.7 (C-17); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3095, 3001, 2919, 2852, 1697, 1574, 1517, 1503, 1471, 1442, 1394, 1352, 1284, 1220, 1184, 1120, 1050, 1004, 981, 957, 881, 794, 760, 547; HRMS (ESI^+): *m/z* calcd. for $C_{17}H_{25}NO_5 + H^+$: 324.1805, found 324.1810.

***rac*-1-(4-Methoxy-2-nitro-5-octanoxyphenyl)ethane-1-ol (5):** The synthesis was done according to general procedure 1; 0.79 g (2.5 mmol, 1.0 equiv.) of ketone **29** was dissolved in 40 mL THF/MeOH, 1:1 and 0.33 g (8.6 mmol, 3.5 equiv.) sodium borohydride was added. Yield: 0.78 g (2.4 mmol, 98 %) as a yellow solid. R_f = 0.23 (CH_2Cl_2); m.p. 42 °C; ^{1}H-NMR (300 MHz, [D_6]DMSO): δ = 7.52 (s, 1 H, H-Ar), 7.34 (s, 1 H, H-Ar), 5.44 (d,$^3J_{H,H}$ = 4.5 Hz, 1 H, OH), 5.26 (dq, $^3J_{H,H}$ = 4.6 Hz, $^3J_{H,H}$ = 6.2 Hz, 1 H, Ph-C*H*-CH_3), 4.12–4.06 (m,2 H, CH_2), 3.84 (s, 3 H, OMe), 1.75 (p, $^3J_{H,H}$ = 7.0 Hz, 2 H, CH_2), 1.45–1.38 (m, 2 H, CH_2), 1.36 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 1.31–1.26 (m, 8 H, 4 × CH_2), 0.86 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, CH_3); ^{13}C-NMR (75 MHz, [D_6]DMSO): δ = 152.7 (C-Ar), 147.2 (C-Ar), 138.7 (C-Ar), 137.9 (C-Ar), 109.7 (C-Ar), 107.4 (C-Ar), 68.6 (CH_2), 63.9 (Ph-*C*-OH), 56.0 (OMe), 31.2, 28.7, 28.6, 28.4, 25.3, 22.0 (6 × CH_2), 25.1 (CH_3), 13.9 (CH_3); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3301, 2926, 2854, 1613, 1579, 1513, 1465, 1445, 1388, 1366, 1329, 1264, 1214, 1166, 1094, 1031, 1012, 896, 797, 723, 658; HRMS (ESI^+): *m/z* calcd. for $C_{17}H_{27}NO_5^+ - H_2O$: 308.1862, found 308.1858.

Bis(1-(4-methoxy-2-nitro-5-octanoxyphenyl)ethyl)phosphonate (12): The synthesis was done according to general procedure 3; 0.43 g (1.3 mmol, 2.1 equiv.) of alcohol **4** was dissolved in 10 mL pyridine and 0.12 mL (0.15 g, 0.65 mmol, 1.0 equiv.) diphenylphosphite was added. Purification was done by column chromatography: PE/EtOAc, 3:1 to PE/EtOAc, 2:1. Yield: 0.33 g (0.47 mmol, 72 %) as a yellow oil and a mixture of four diastereomers. No single isomer was obtained pure, provided NMR-data is given for the obtained diastereomeric mixture. R_f = 0.30 (PE/EtOAc, 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.57 (s, 2 H, H-Ar, 4 × *ds*), 7.11 (s, 2 H, H-Ar, 4 × *ds*), 6.37 (d, $^1J_{H,P}$ = 703 Hz, 1 H, P-H, 2 × *ds*), 6.29–6.11 (m, 2 H, Ar-C*H*-P), 4.06–3.96 (m, 8 H, CH_2), 3.93 (s, 3 H, OMe, 4 × ds), 1.90–1.83 (m, 8 H, CH_2), 1.66 (d, $^3J_{H,H}$ = 6.1 Hz, 3 H, CH_3, 4 × ds), 1.52–1.44 (m, 8 H, CH_2), 1.41–1.26 (m, 32 H, 8 × CH_2), 0.90–0.87 (m, 12 H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 153.8, 153.6 (C-5, *ds*), 148.6, 148.5, 148.3, 148.2 (C-Ar, *ds*), 138.7, 138.5, 138.2, 138.1 (C-Ar, *ds*), 132.2, 132.1, 132.0, 132.0, (C-Ar, *ds*), 108.7, 108.7, (C-Ar, *ds*), 107.8, 107.8, (C-Ar, *ds*), 70.9 (CH_2), 69.7, 69.6 (d, $^2J_{C,P}$ = 2.8 Hz, Ar-*C*-P, *ds*), 56.4, 56.3, 56.3, 56.2, (OMe, *ds*),29.4–29.3, 28.9 (d, $^2J_{C,P}$ = 3 Hz, CH_3, *ds*), 29.2, 25.9, 24.9, 24.8, 24.6, 24.6, (CH_2, *ds*), 14.2, 14.1, (CH_3, *ds*); ^{31}P-NMR (162 MHz, $CDCl_3$): δ = 5.27, 4.56, 4.27; IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2926, 2854, 1579, 1515, 1465, 1442, 1376, 1268, 1216, 1173, 1104, 1036, 944, 797, 722, 624, 522; HRMS (ESI^+): *m/z* calcd. for $C_{34}H_{53}N_2O_{11}P + Na^+$: 719.3285, found 719.3377.

γ-Bis(2-nitrobenzyl)ATP (13): The synthesis was done according to general procedure 4; 0.24 g (0.23 mmol, 1.0 equiv.) ADP **24**, 0.13 g (0.30 mmol, 1.3 equiv.) of phosphoramidite **6** were dissolved in 10 mL dry CH_3CN and 0.08 g (0.35 mmol, 1.5 equiv.) pyridinium trichloroacetate and 64 μL (0.35 mmol, 1.5 equiv.) *tert*-butylhydroperoxide (5.5 M, in *n*-decan) were added. Yield: 0.13 g (0.16 mmol, 72 %) as a yellow cotton. Yield was calculated with two ammonium counterions. ^{1}H-NMR (600 MHz, D_2O): δ = 8.40 (s, 1 H, H-Ar), 8.11 (s, 1 H, H-Ar), 7.98–7.93 (m, 2 H, H-Ar), 7.56–7.53 (m, 2 H, H-Ar), 7.53–7.48 (m, 2 H, H-Ar), 7.44–7.41 (m, 2 H, H-Ar), 5.89 (d, $^3J_{H,H}$ = 5.5 Hz, 1 H, H-1′), 5.41 (dd, $^3J_{H,P}$ = 8.3 Hz, $^2J_{HH}$ = 2.2 Hz, 2 H, Ph-CH_2-a), 5.37 (d, $^3J_{H,P}$ = 8.3 Hz, 2 H, Ph-CH_2-b), 4.60–4.57 (m, 1 H, H-2′), 4.46 (dd, $^3J_{H,H}$ = 5.2 Hz, $^3J_{H,H}$ = 3.7 Hz, 1 H, H-3′), 4.33–4.28 (m, 2 H, H-4′, 5′a), 4.26–4.20 (m, 1 H, H-5′b); ^{13}C-NMR (150 MHz, D_2O): δ = 153.5 (C-Ar), 148.9 (C-Ar), 148.0 (C-Ar), 146.0 (C-Ar), 145.8 (C-Ar), 140.5 (C-Ar), 134.5, 134.4 (C-Ar, *rotamer*), 130.7, 130.6 (d, $^3J_{C,P}$ = 4.7 Hz, *rot.*), 129.3, 129.2 (C-Ar, *rot*), 128.9, 128.6 (C-Ar, *rot.*), 124.9, 124.8 (C-Ar, *rot.*), 118.0 (C-Ar), 87.2 (C-1′), 83.6 (d, $^3J_{C,P}$ = 9.4 Hz, C-4′), 74.6 (C-2′), 70.0 (C-3′), 66.9 (m, Ph-CH_2), 65.9 (m, C-5′); ^{31}P-NMR (242 MHz, D_2O): δ = –10.5 (d, $^2J_{P,P}$ = 20.5 Hz, P-α), –11.9 (d, $^2J_{P,P}$ = 17.0 Hz, P-γ), –23.0 (dd, $^2J_{P,P}$ = 20.6 Hz, $^2J_{P,P}$ = 16.9 Hz, P-β); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3047, 1689, 1641, 1608, 1523, 1445, 1340, 1237, 1127, 1080, 1020, 993, 921, 815, 726, 462; HRMS (ESI^+): *m/z* calcd. for $C_{24}H_{26}N_7O_{17}P_3 + H^+$: 778.0676, found 778.0714.

γ-Bis(4,5-dimethoxy-2-nitrobenzyl)ATP (14): The synthesis was done according to general procedure 4; 0.13 g (0.23 mmol, 1.5 equiv.) of phosphoramidte **7** and 0.15 g (0.16 mmol, 1.0 equiv.) ADP **24** were dissolved in 10 mL dry CH_3CN and 0.94 mL (0.23 mmol, 1.5 equiv.) DCI-activator solution (0.25 M in CH_3CN) and

0.48 μL (0.27 mmol, 1.7 equiv.) *tert*-butylhydroperoxide (5.5 M, in *n*-decan) were added. Yield: 89 mg (96 μmol, 60 %) as a yellow cotton. Yield was calculated with two ammonium counterions. ^{1}H-NMR (600 MHz, D_2O): δ = 8.40 (s, 1 H, H-Ar), 8.14 (s, 1 H, H-Ar), 7.40 (s,1 H, H-Ar), 7.38 (s,1 H, H-Ar), 6.80 (s,1 H, H-Ar), 6.73 (s, 1 H, H-Ar), 5.86 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H, H-1′), 5.27–5.18 (m, 4 H, Ph-CH_2), 4.54 (*pt*, 1 H, H-2′), 4.46 (*pt*, 1 H, H-3′), 4.38–4.33 (m, 1 H, H-5′a), 4.33–4.30 (m, 1 H, H-4), 4.28–4.22 (m, 1 H, H-5′), 3.82 (s, 6 H,2 × OMe), 3.77 (s, 6 H,2 × OMe); ^{13}C-NMR (150 MHz, D_2O): δ = 152.7 (C-Ar), 152.6, (C-Ar), 152.1 (C-Ar), 148.4 (C-Ar), 147.4 (C-Ar), 140.5 (C-Ar), 138.5 (d, $^3J_{C,P}$ = 12 Hz, C-Ar), 119.1 (C-Ar), 117.8 (C-Ar), 110.5 (C-Ar), 110.4 (C-Ar), 107.5 (C-Ar), 107.5 (C-Ar), 87.1 (C-1′), 83.6 (C-4′), 74.5 (C-2′),69.3 (C-3′), 66.8 (Ph-CH_2), 64.8 (C-5′), 56.2, 56.2, 55.9 (OMe); ^{31}P-NMR (242 MHz, D_2O): δ = –11.9 (d, $^2J_{P,P}$ = 20.5 Hz, P-α), –13.3 (d, $^2J_{P,P}$ = 16.9 Hz, P-γ), –24.0 to –24.3 (m, P-β); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3141, 1679, 1521, 1327, 1275, 1218, 1169, 1127, 1066, 1019, 924, 813, 753, 710, 604; HRMS (ESI^-): *m/z* calcd. for $C_{28}H_{33}N_7O_{21}P_3^-$: 896.0948, found 896.0648.

γ-Bis(5-methoxy-2-nitro-4-octanoxybenzyl)ATP (15): The synthesis was done according to general procedure 4; 0.16 g (0.21 mmol, 1.5 equiv.) of phosphoramidite **8** and 0.13 g (0.14 mmol, 1.0 equiv.) ADP **24** were dissolved in 10 mL dry CH_3CN and 0.85 mL (0.21 mmol, 1.5 equiv.) DCI-activator solution (0.25 M in CH_3CN) and 0.44 μL (0.24 mmol, 1.7 equiv.) *tert*-butylhydroperoxide (5.5 M, in *n*-decan) were added. Yield: 50 mg (45 μmol, 38 %) as a yellow cotton. The yield was calculated with two ammonium counterions. ^{1}H-NMR (600 MHz, [D_4]MeOD): δ = 8.65 (s, 1 H, H-Ar), 8.20 (s, 1 H, H-Ar), 7.55 (s, 1 H, H-Ar), 7.54, (s, 1 H, H-Ar), 7.25 (s, 1 H, H-Ar), 7.24(s, 1 H, H-Ar), 5.99 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H, H-1′), 5.57–5.46 (m, Ph-C*H*$_2$), 4.50–4.45 (m, 2 H, H-2′, 3′), 4.40–4.36 (m, 1 H, H-5′a), 4.30 (ddd, $^3J_{H,H}$ = 11.5, $^2J_{H,P}$ = 6.0, $^2J_{H,H}$ = 2.4 1 H, H-5′b), 4.24–4.21 (m, 1 H, H-4′), 4.00–3.97 (m, 4 H, 2 × CH_2), 3.91 (s, 3 H, OMe), 3.90 (s, 3 H, OMe), 1.80 (p, $^3J_{H,H}$ = 7.6 Hz, 4 H, 2 × CH_2), 1.49 (p, $^3J_{H,H}$ = 7.1 Hz, 4 H, 2 × CH_2), 1.42–1.27 (m, 16 H, 8 × CH_2), 0.91 (t, $^3J_{H,H}$ = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C-NMR (150 MHz, [D_4]MeOD): δ = 155.5 (C-Ar), 153.6 (C-Ar), 149.2 (C-Ar), 147.6 (C-Ar), 140.7 (C-Ar), 138.7 (C-Ar), 126.8 (C-Ar), 111.8 (C-Ar), 111.7 (C-Ar), 110.8 (C-Ar), 110.0 (C-Ar), 90.0 (C-1′), 85.4 (d, $^3J_{C,P}$ = 9.6 Hz, C-4′), 76.6 (C-2′), 71.1 (C-3′), 70.5 (d, $^2J_{C,P}$ = 9.6 Hz, Ph-CH_2), 68.0 (CH_2), 65.4 (d, $^2J_{C,P}$ = 4.1 Hz, C-5′), 57.2, 56.8 (2 × OMe), 33.0, 30.5, 30.4, 30.1, 27.1, 23.7 (6 × CH_2), 14.5 (CH_3); ^{31}P-NMR (262 MHz, [D_4]MeOD): δ = –11.8 (d, $^2J_{P,P}$ = 20.4 Hz, P-α), –13.3 (d, $^2J_{P,P}$ = 16.8 Hz, P-γ), –20.4 (dd, $^2J_{P,P}$ = 20.4 Hz, $^2J_{P,P}$ = 16.5 Hz, P-β); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3191, 2924, 2854, 1578, 1520, 1463, 1326, 1275, 1217, 1128, 1067, 991, 872, 811, 754, 639; HRMS (ESI^-): *m/z* calcd. for $C_{42}H_{61}N_7O_{21}P_3^-$: 1092.3139, found 1092.3075.

γ-Bis-*O*-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)ATP (16): The synthesis was done according to general procedure 5; 0.20 g (0.40 mmol, 1.0 equiv.) of *H*-phosphonate **11** was activated with 0.11 g (0.80 mmol, 2.0 equiv.) *N*-chlorosuccinimide and 0.31 g (0.30 mmol 0.8 equiv.) ADP **24** was added. Yield: 0.14 g (0.15 μmol, 49 %) as a yellow cotton in a mixture of four diastereomers. The yield was calculated with two ammonium counterions. ^{1}H-NMR (600 MHz, [D_4]MeOD): δ = 8.65, 8.64, 8.63, 8.61, (s, 1 H, H-Ar, *ds.*), 8.20, 8.19, 8.19 (s, 1 H, H-Ar, *ds.*), 7.43–7.29 (m, 2 H, H-Ar), 7.16–7.04 (m, 2 H, H-Ar), 6.15–6.02 (m, 2 H, H-1′, Ph-C*H*-P), 4.65–4.59 (m, 1 H, H-2′), 4.53–4.49 (m, 1 H, H-4′), 4.36–4.30 (m, 2 H, H-3′, 5′a), 4.29–4.21 (m, 1 H, H-5′b), 3.90, 3.88, 3.87, 3.83 (s, 12 H, OMe, *ds.*), 1.66–1.51 (m, 6 H, 2 × CH_3); ^{13}C-NMR (150 MHz, D_2O): δ = 154.1, 153.9, 153.7 (C-Ar, *ds*), 148.2, 148.2, 148.0, 147.9 (C-Ar, *ds*), 138.3, 138.1, 137.9 (C-Ar, *ds*), 132.7–132.6, 132.5–132.4, 132.1–131.9 (3 × m, C-Ar, *ds*), 108.7, 108.7, 108.3, 107.6, 107.2, 107.1, 107.0, 106.8 (C-Ar, *ds*), 88.1, 88.0, 87.9 (C-1′, *ds*), 84.1, 84.1 (C-4′, *ds*), 75.2, 75.1, 75.1 (C-2′, *ds*), 73.6–73.4, 73.0–72.8, 72.8–72.7 (3 × m, Ph-C*H*-O), 70.3, 70.2, 70.1 (C-3′,*ds*), 65.0–64.9 (m, C-5′, *ds*), 55.9–55.8, 55.7, 55.5, 55.5, 55.4–55.3, 54.1–55.1 (OMe, *ds*), 23.4–22.9 (CH_3, *ds*); ^{31}P-NMR (242 MHz, [D_4]MeOD): δ = –11.6 (d, $^2J_{P,P}$ = 19.2 Hz, P-α), –11.7 (d, $^2J_{P,P}$ = 20.8 Hz, P-α), –14.1 (d, $^2J_{P,P}$ = 17.9 Hz, P-γ), –15.5 (d, $^2J_{P,P}$ = 16.9 Hz, P-γ), –23.3 to –23.8 (m, P-β); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3175, 1642, 1580, 1518, 1453, 1379, 1272, 1218, 1172, 1127, 1077, 922, 874, 816, 794, 711, 642; HRMS (ESI^-): *m/z* calcd. for $C_{30}H_{38}N_7O_{21}P_3^-$ + H^+: 926.1412, found 926.1443.

γ-Bis-*O*-(1-(4-methoxy-2-nitro-5-octanoxyphenyl)ethyl)ATP (17): The synthesis was done according to general procedure 4b; 0.16 g (0.23 mmol, 1.0 equiv.) of *H*-phosphonate **11** was activated with 62 mg (0.46 mmol, 2.0 equiv.) *N*-chlorosuccinimide and 0.23 g (0.20 mmol 0.8 equiv.) ADP **24** was added. Yield: 53 mg (46 μmol, 23 %) as a yellow cotton in a mixture of four diastereomers. The yield was calculated with two ammonium counterions. ^{1}H-NMR (600 MHz, [D_4]MeOD): δ = 8.63, 8.62, 8.62, 8.60 (s, 1 H, H-Ar, *ds.*), 8.19, 8.18 (s, 1 H, H-Ar, *ds.*), 7.40–7.36 (m, 2 H, H-Ar), 7.06–6.93 (m, 2 H, H-Ar), 6.16–6.06 (m, 2 H, H-1′, Ph-C*H*-P, *ds.*), 4.69–4.64 (m, 1 H, H-2′), 4.55–4.51 (m, 1 H, H-4′), 4.35–4.23 (m, 3 H, H-3′, 5′), 4.00–3.91 (m, 4 H, 2 × CH_2), 3.91, 3.89, 3.87 (s, 6 H, 2 × OMe, *ds.*), 1.85–1.77 (m, 4 H, 2 × CH_2), 1.64–1.53 (m, 6 H, 2 × CH_3), 1.52–1.44 (m, 4 H, 2 × CH_2), 1.42–1.27 (m, 16 H, 8 × CH_2), 0.94–0.88 (m, 6 H, 2 × CH_3); ^{13}C-NMR (150 MHz, [D_4]MeOD): δ = 155.1, 155.1, 154.9 (C-Ar, *ds*), 153.4 (C-Ar, *ds*), 149.6, 149.2 (C-Ar, *ds*), 148.0 (C-Ar), 137.9 (C-Ar), 131.9 (C-Ar), 110.5, 110.1, 110.1 (C-Ar, *ds*), 108.6, 108.3 (C-Ar, *ds*), 91.2 (C-1′), 83.7 (C-4′), 76.5 (C-2′), 73.9 (Ph-C*H*-O), 71.0 (C-3′), 70.0–69.9 (m, C-5′), 65.9 (CH_2), 56.7, 56.6, 56.5 (OMe, *ds*); 33.0, 30.6–30.1, 27.3–27.0, 23.7 (CH_2), 24.7 (d, $^3J_{C,P}$ = 4.5 Hz, CH_3), 24.6 (d, $^3J_{C,P}$ = 4.4 Hz, CH_3), 23.8 (d, $^3J_{C,P}$ = 4.3 Hz, CH_3), 14.5 (CH_3); ^{31}P-NMR (242 MHz, [D_4]MeOD): δ = –11.5 to –11.9 (m, P-α), –13.5 to –13.6 (m, P-γ), –15.5 (d, $^2J_{P,P}$ = 15.4 Hz, P-γ), –17.2 (d, $^2J_{P,P}$ = 16.5 Hz, P-γ), –23.4 to –23.9 (m, P-β); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3191, 2926, 2854, 1580, 1519, 1442, 1379, 1329, 1271, 1217, 1175, 1104, 1079, 994, 871, 818, 757, 720, 624, 492; HRMS (ESI^-): *m/z* calcd. for $C_{44}H_{65}N_7O_{21}P_3^-$: 1120.3452, found 1120.3397.

4-(Hydroxymethyl)-phenyldecanoate (19): The synthesis of compound **7** was performed as described in the literature. The analytical data is in accordance with the literature.[25]

***O*-(1-(4,5-Dimethoxy-2-nitrophenyl)ethyl)-bis(*N*,*N*-diisopropylamin)phosphoramidite (20):** Under anhydrous conditions a solution containing 0.30 g (1.3 mmol, 1.2 equiv.) of alcohol **4** and 0.45 mL (0.33 g, 3.3 mmol, 3.0 equiv.) anhydrous triethylamine in 10 mL anhydrous THF were added dropwise to an ice-cold solution of 0.29 g (1.1 mmol, 1.0 equiv.) bis(*N*,*N*-diisopropylamino)-chlorophosphine in 10 mL anhydrous THF over a period of 30 minutes. The resulting mixture was stirred at room temperature for 18 h. The precipitate was removed and the filtrate was concentrated to dryness in vacuo. The remaining residue was purified on silica gel (PE/EtOAc, 5:1 + 5 % TEA). Yield: 0.44 g (0.97 mmol, 88 %) as a yellow solid. R_f = 0.50 (PE/EtOAc, 5:1 + 5 % TEA); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.56 (s, 1 H, H-Ar), 7.39 (s, 1 H, H-Ar), 5.51 (dq, $^3J_{H,P}$ = 12.4 Hz, $^3J_{H,H}$ = 6.2 Hz, 1 H, Ph-C*H*-O), 3.96 (s, 3 H, OMe), 3.93 (s, 3 H, OMe), 3.63–3.39 (m, 4 H, PNC-*H*), 1.53 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 1.20 (4 × d, $^3J_{H,H}$ = 6.6 Hz, 6 H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 153.7 (C-Ar), 147.5 (C-Ar), 139.4 (C-Ar), 138.1 (d, $^3J_{C,P}$ = 2.1 Hz, C-Ar), 109.6 (C-Ar), 107.4 (C-Ar), 66.9 (d, $^2J_{C,P}$ = 17.7 Hz, Ph-CH_2-P), 56.4, 56.3 (2 × OMe), 44.9, 44.7 (d, $^2J_{C,P}$ = 12.6 Hz, PNC), 25.2 (d, $^3J_{C,P}$ = 3.6 Hz, CH_3), 24.5–24.3 (m, CH_3); ^{31}P-NMR (162 MHz, $CDCl_3$): δ = 148.8; IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2966, 2929, 2868, 1519, 1497, 1462, 1452, 1438, 1319, 1272, 1219, 1184, 1114, 1099, 1051, 951, 916, 873, 794, 759, 672, 525; HRMS (ESI^-): *m/z* calcd. for $C_{22}H_{40}N_3O_5P$ + H^+: 474.2733, found 474.2730.

(1-(4,5-Dimethoxy-2-nitrophenyl)ethyl)-(4-decanoyloxybenzyl)-phosphonate (23): Under anhydrous conditions 0.34 mL (0.41 g, 1.8 mmol, 1.0 equiv.) diphenylphosphite were dissolved in 20 mL dry pyridine, cooled to −15 °C and mixed with 0.40 g (1.8 mmol, 1.0 equiv.) of compound **4**. The solution was stirred for 45 min at −15 °C, then 0.73 g (2.6 mmol, 1.5 equiv.) of compound **19** was added stepwise. After complete addition, the reaction mixture was stirred for two hours at room temperature before all volatile compounds were removed in vacuo. The residue was coevaporated first with toluene and afterward with CH_2Cl_2. The residue was purified by column chromatography on silica gel (PE/EtOAc, 1:1). Yield: 0.38 g (0.69 mmol, 38 %) as a yellow oil and a mixture of four diastereomers. The provided NMR data is given for the dominant diastereomer. R_f = 0.20 (PE/EtOAc, 1:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.59 (s, 1 H, H-Ar), 7.34–7.28 (m, 2 H, H-Ar), 7.09–7.00 (m, 2 H, H-Ar), 7.14 (s, 1 H, H-Ar), 6.95 (d, $^1J_{H,P}$ = 705 Hz, 1 H, P-H), 6.35–6.20 (m, 1 H, Ph-C*H*), 5.08–4.98 (m, 2 H, Ph-CH_2), 4.01–3.98 (m, 6 H,2 × OMe), 2.55 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH_2), 1.74 (p, $^3J_{H,H}$ = 7.4 Hz, 2 H, CH_2), 1.65 (m, 3 H, CH_3), 1.45–1.11 (m, 12 H, 6 × CH_2), 0.88 (t, $^3J_{H,H}$ = 6.7 Hz, 3 H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 172.3 (C-Ar), 154.0 (C-Ar), 151.2 (C-Ar), 148.4 (C-Ar), 139.8 (C-Ar), 132.8 (C-Ar), 129.3 (C-Ar), 122.1 (C-Ar), 108.7 (C-Ar), 107.8 (C-Ar), 71.2 (Ph-*C*H), 67.0 (d, $^2J_{C,P}$ = 8.7 Hz, Ph-*C*H_2), 56.5 (2 × OMe), 34.5 (CH_2), 32.0 (CH_2), 25.0 (CH_2), 24.7 (d, $^3J_{C,P}$ = 4.9 Hz, CH_3), 14.2 (CH_3); ^{31}P-NMR (162 MHz, $CDCl_3$): δ = 8.9, 8.9; IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2925, 2853, 1756, 1580, 1517, 1461, 1375, 1330, 1271, 1166, 1104, 1020, 947, 872, 815, 793, 758, 558; HRMS (ESI$^+$): *m/z* calcd. for $C_{27}H_{38}NO_9P + Na^+$: 574.2182, found 574.2186.

(1-(4,5-Dimethoxy-2-nitrophenyl)ethyl)-(4-decanoyloxybenzyl)-diisopropylphosphoramidite (21): The synthesis is based on general procedure 4; 0.16 g (0.21 mmol, 1.5 equiv.) of alcohol **19** and 0.33 g (0.72 mmol, 1.3 equiv.) of diamidite **20** was dissolved in 15 mL dry THF, cooled to 0 °C and 1.8 mL (0.48 mmol, 1.0 equiv.) DCI-activator solution (0.25 M in CH_3CN) was added. The solution was stirred for one hour at room temperature. All volatile compounds were removed in vacuo and the remaining residue was purified by column chromatography on silica gel (PE/EtOAc, 5:1 + 5 % TEA). Yield: 0.29 g (0.45 mmol, 93 %) as a yellow oil and a mixture of four diastereomers. The provided NMR data is given for the dominant diastereomer. R_f = 0.75 (PE/EtOAc, 5:1 + 5 % TEA); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.56 (s, 1 H, H-Ar), 7.38–7.31 (m, 3 H, H-Ar), 7.08–7.01 (m, 2 H, H-Ar), 5.80–5.67 (m, 1 H, Ph-C*H*), 4.80–4.47 (m, 2 H, PH-CH_2), 3.95–3.90 (m, 6 H, 2 × OMe), 3.63–3.44 (m, 2 H, PNC*H*), 2.59–2.48 (m, 2 H, CH_2), 1.81–1.67 (m, 2 H, CH_2), 1.67–1.49 (m, 5 H, CH_2, CH_3), 1.47–1.24 (m, 10 H, 5 × CH_2), 1.22–0.96 (m, 12 H, CH_3), 0.91–0.81 (m, 2 H, H-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 172.5 (C-Ar), 153.7 (C-Ar), 150.0 (C-Ar), 147.7 (C-Ar), 139.0 (C-Ar), 137.0 (C-Ar), 128.8 (C-Ar), 128.1 (C-Ar), 121.6 (C-Ar), 109.5 (C-Ar), 107.4 (C-Ar), 67.8 (PhCH), 65.2 (Ph-CH_2), 56.5, 56.5 (OMe), 43.2 (d, $^2J_{C,P}$ = 12.5 Hz, PNC), 34.5 (CH_2), 24.8 (CH_2), 24.8 (CH_3), 29.6, 29.4, 29.3 (CH_2), 25.1 (C-CH_3), 14.3 (CH_3); ^{31}P-NMR (162 MHz, $CDCl_3$): δ = 145.6, 144.5; IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 2964, 2927, 2854, 1758, 1580, 1518, 1460, 1394, 1364, 1332, 1271, 1198, 1183, 1164, 1103, 1012, 971, 919, 874, 793, 756, 597, 564; HRMS (ESI$^-$): Could not be detected neither in positive or negative mode.

γ-(*O*-1-(4,5-Dimethoxy-2-nitrophenyl)ethyl)-(4-decanoyloxybenzyl)-ATP (22): The synthesis was done according to general procedure 5; 55 mg (0.10 mmol, 1.0 equiv.) of *H*-phosphonate **23** was activated with 27 mg (0.20 mmol, 2.0 equiv.) *N*-chlorosuccinimide and 0.08 g (0.08 mmol, 0.8 equiv.) ADP **24** was added. Yield: 24 mg (20 μmol, 26 %) as a yellow cotton in a mixture of four diastereomers. ^{13}C-signals were obtained from HSQC experiment, due to the low amount of substance. ^{1}H-NMR (400 MHz, [D_4]MeOD): δ = 8.64, 8.64, (s, 1 H, H-Ar, *dia.*), 8.18 (s, 1 H, H-Ar), 7.55–7.45 (m, 1 H, H-Ar), 7.36–7.18 (m, 3 H, H-Ar), 7.03–6.89 (m, 2 H, H-Ar), 6.28–6.16 (m, 1 H, Ph-C*H*), 6.07–6.02 (m, 1 H, H-1′), 5.20–5.07 (m, 2 H, H-2′, 3′ H), 4.60–4.52 (m, 1 H, H-4′), 4.49–4.40 (m, 1 H, H-5′a), 4.35–4.18 (m, 3 H, H-5′b, Ph-CH_2), 3.93, 3.86, 3.81, (s, 6 H, OMe, *ds.*), 2.61–2.51 (m, 2 H, CH_2), 1.77–1.70 (m, 2 H, CH_2), 1.70–1.57 (m, 3 H, CH_3, *ds.*), 1.46–1.25 (m, 12 H, 6 × CH_2), 0.90 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_3); ^{13}C-NMR (150 MHz, [D_4]MeOD): δ = 128.1 (C-Ar, *ds*), 120.9 (C-Ar, *ds*), 108.0 (C-Ar, *ds*), 106.6 (C-Ar), 87.4 (C-1′), 83.6 (C-4′), 74.9 (C-2′), 72.7 (Ph-*C*H-O), 69.9 (C-3′), 64.7 (m, C-5′), 68.4 (CH_2), 55.0 (OMe, *ds*), 33.5, 29.1, 29.1, 29.0, 24.5, 22.2 (7 × CH_2) 24.6 (d, $^3J_{C,P}$ = 4.4 Hz, CH_3), 22.2 (Ph-CH-*C*H_3), 14.5 (CH_3); ^{31}P-NMR (162 MHz, [D_4]MeOD): δ = -11.0 to −11.3 (m, P-α). −13.3 to −14.0 (m, P-γ). −22.7 to −23.1 (m, P-γ); IR (ATR): $\tilde{\nu}$ = [cm^{-1}] = 3175, 2928, 2854, 2684, 1673, 1606, 1579, 1457, 1377, 1326, 1234, 1169, 1129, 1070, 1020, 991, 902, 810, 718; HRMS (ESI$^-$): *m/z* calcd. for $C_{37}H_{50}N_6O_9P^-$: 975.2349, found 975.2280.

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