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Traceless Staudinger acetylation of azides in aqueous buffers

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ABSTRACT

In this paper, we demonstrate the applicability of water-soluble *p*-dimethylaminoethyl substituted phosphinomethanethiol in acetyl transfer reactions by the traceless Staudinger ligation with unprotected ε-azido lysine containing peptides in aqueous buffer systems. Additionally, we present an improved synthesis pathway for the water-soluble phosphinothiol linkers requiring less steps in a comparable overall yield in comparison to previously published protocols.

1. Introduction

The human genome has about 20,000 genes to code all different proteins that control our daily life on a cellular level.[1] Since this number is not sufficient to undertake all the different functions in the body, nature employs, in addition to RNA splicing events, several enzymatic posttranslational modifications, such as acetylation, prenylation, glycosylation, methylation and phosphorylation, which add additional levels of complexity to the protein level.[2] These apparently small molecular changes can have a big impact on the protein's specific function by influencing its overall structure and can lead to activation or deactivation of enzymes or other cellular processes.[2] Recently, many proteins have been discovered in the cytoplasm of eukaryotes and prokaryotes that carry different lysine side chain modulations, such as acetylation or methylation, but their specific role is only partially understood.[3] For example, histone proteins—found in the nuclei as part of the chromatin—apply these posttranslational modifications on lysines to alter the chromatin's nucleosomal structure. These changes were shown to be critical in epigenetic processes such as gene repression or activation controlling the transcription of DNA and thereby the evolution of life.[3]

In order to access proteins that are posttranslationally modified at selected lysine residues, several strategies have been developed by chemical biologists over the last years. These approaches include the use of Native Chemical Ligation (NCL) for the introduction of lysine derivatives in the N-terminal histone tail,[4] enzymatic lysine acetylation[5] and amber suppression that enables selective incorporation of acetyl lysine by employing an aminoacyl-tRNA synthetase and $tRNA_{CUA}$ pair created by direct evolution.[6] Another common approach for the access of site-specifically modified biopolymers is the introduction of non-native functional groups, so-called bioorthogonal reporters, into biopolymers that can chemically react in a selective fashion without protection of distal naturally occurring functional groups.[7] Recent studies often involve the application of an azide group as to date, several well explored methods for its incorporation into a biomolecule exist.[8] The ready access to proteins carrying unnatural functional groups is complimented by the engineering of several synthetic transformations during the last years that use proteins as substrates for mild and chemoselective organic reactions.[7] In addition to using these reactions for the attachment of functional modules, such as purification tags or fluorescent labels, several studies were devoted to mimic enzymatic protein modifications by chemical transformations.[9] For purpose of installing acetylated lysine side chains in proteins, a thiol-ene based labeling strategy that enables selective conversion of cysteines to acetyl lysine derivatives on proteins, was published recently,[10] which is only limited by the presence of additional cysteines in the protein.

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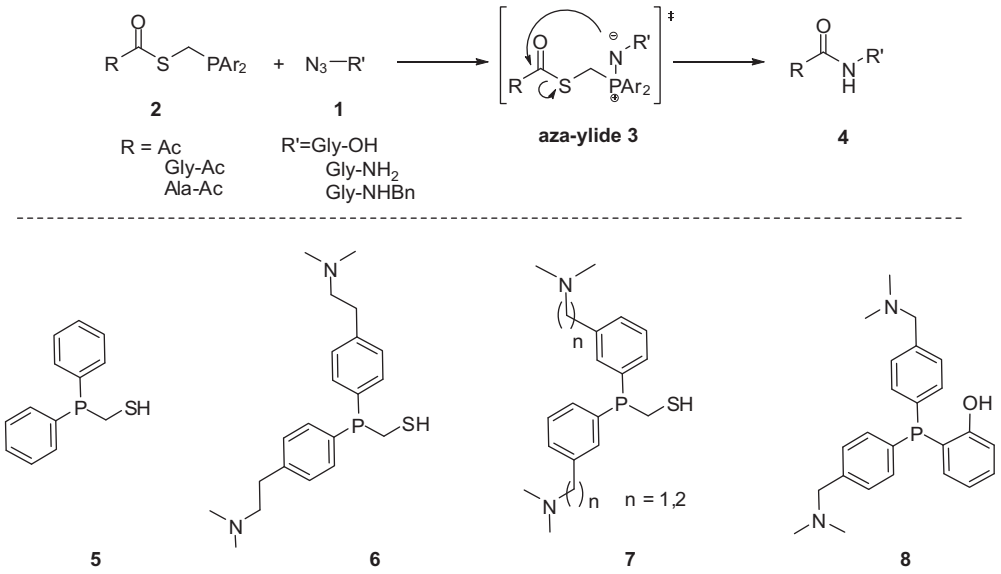


Scheme 1. Traceless Staudinger ligation with water-soluble phosphines and different phosphine linker systems **5**–**8**.

In this paper, we aim to develop an alternative for the purpose of installing acetylated lysine side chains in unprotected peptides by a chemical modification reaction. We chose the traceless Staudinger ligation for the conversion of ε-azido-lysine residues (Scheme 1).[11] The traceless Staudinger ligation—first developed independently by Raines[12] and Bertozzi[13]—is based on the Staudinger reaction,[14] in which an azide **1** reacts with a phosphine **2** via a four-membered cyclic transition state to an aza-ylide **3**. Instead of a P=N bond hydrolysis in the Staudinger reduction to yield an amine and a phosphine oxide, a (thio-)ester was introduced as an electrophilic trap to enable an intramolecular shift and amide bond formation to **4** under the release of phosphine oxide.[15] Among the few designed phosphine linkers, (diphenylphosphino-)methanethiol (**5**) has shown great potential and practical applicability towards small molecules,[16] but its main shortcoming was its weak solubility in aqueous media required for peptides and proteins. The successive efforts of Raines and his group have resulted in a first family of water-soluble phosphines and opened up new possibilities for ligations in aqueous systems.[17] It was shown that compounds **6** and **7** bearing dimethylamino-substituents give better conversions than negatively charged derivatives, such as carboxylic acids. In addition, studies on coulombic effects depending on the position of the dimethylamino-groups on the two aryl substituents showed a positive impact of *meta*-positioned amino groups in **7**.[17] Subsequently, Marx has presented water-soluble phosphine **8** possessing a phenol core instead of a thiomethylene moiety.[18] Notably, none of the water-soluble variants has to the best of our knowledge been subjected to traceless Staudinger ligations on more complex unprotected peptides or proteins.[19]

In this paper, we first present a modified synthetic route to acetylated bis(*p*-dimethylaminoethyl)-phosphinomethanethiol (**15**), which we intend to probe in acetylation studies of a benzylazide derivative and a more complex azido peptide in aqueous systems. Previously, phosphine **6** was obtained by Raines et al. via the secondary phosphine borane **14** that was further reacted with formaldehyde to give the tertiary hydroxymethyl phosphine borane.[17a] The alcohol was then transformed into the corresponding mesylate and subjected to substitution with potassium thioacetate to yield acetylthioester **15**, which can be hydrolysed to deliver the free thiol **6**. This three-step procedure can be shortened by direct alkylation of the secondary phosphine borane **14** with commercially available acetylthiomethyl bromide.

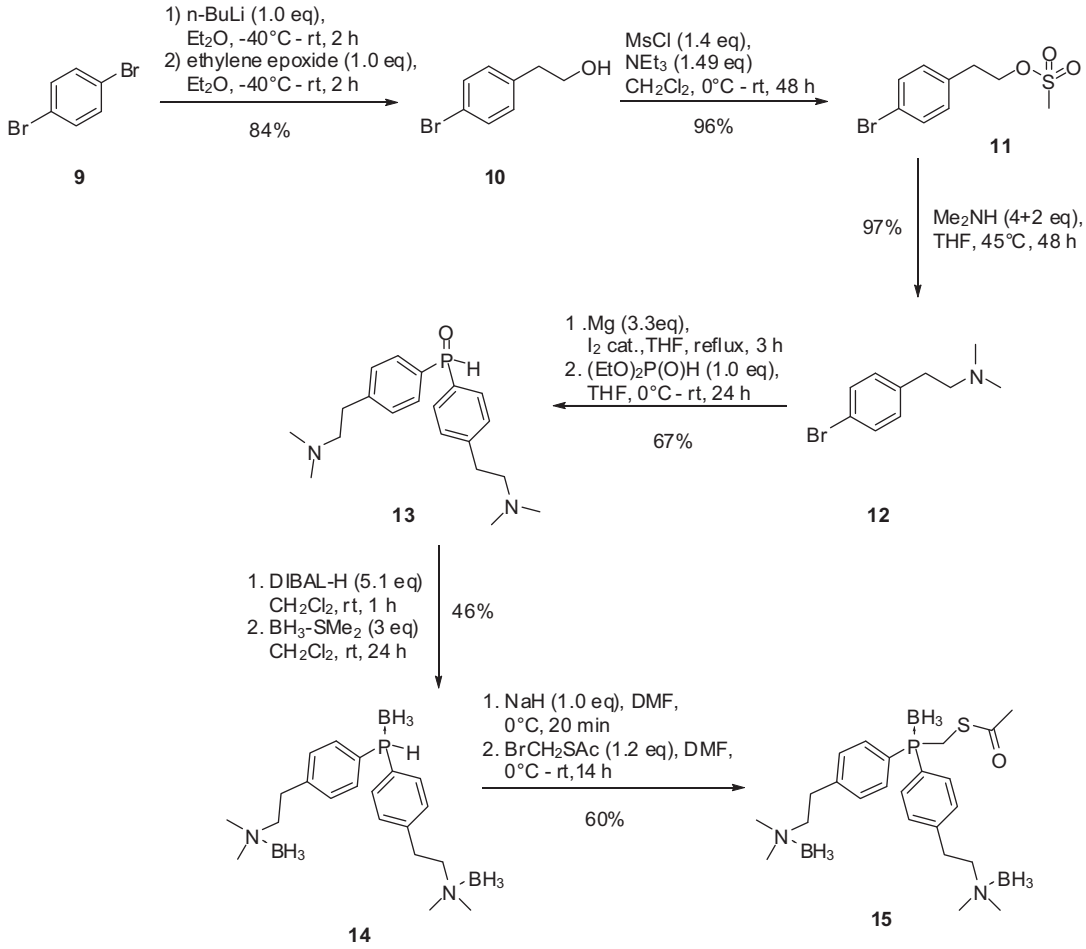
2. Results and discussion

2.1. Phosphine synthesis

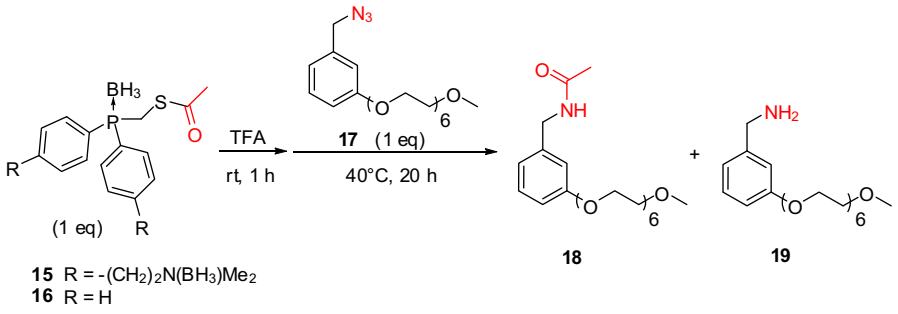
Our synthesis started with a *n*-butyllithium mediated metal–halogen exchange on commercially available 1,4-dibromobenzene **9** and substitution of the formed lithium reagent with ethylene epoxide (Scheme 2). Distillation yielded 85% of pure 4-bromophenylethyl alcohol **10**. The alcohol was converted into mesylate **11** in 96% yield after crystallization and then transformed into amine **12**. By the addition of another portion of dimethylamine (2 equiv) and prolonged reaction times, the conversion of the reaction could be increased from 67% to 97%. The resulting 4-(2-dimethylaminoethyl)bromobenzene **12** was converted into the Grignard reagent and reacted with diethyl phosphite. Phosphine oxide **13** could be isolated in 67% yield. Subsequently **13** was reduced by reaction with an excess of diisobutylaluminum hydride (DIBAL-H). The free phosphine and the amino groups were directly complexed with BH_3 to give the secondary phosphine borane **14** in 46% yield. This one-pot reduction protocol proved to be the most demanding synthetic transformation in this route due to the sensitivity of **14** during isolation. The last step was performed by alkylation of **14** with acetylthiomethyl bromide. The newly developed one-step procedure yielded 60% of the desired product **15**, which corresponds to a similar overall yield of 15%, which was reported by Raines et al. in 2007.[17a,20] In comparison to the previously published synthesis, we reduced the number of reaction steps from seven to five and could increase the yield from **14** to **15** from 46% to 60%.[17a]

2.2. First studies on traceless Staudinger ligation

After the successful synthesis of phosphine borane **15**, we wanted to evaluate its ability to mediate an acetyl transfer by traceless Staudinger ligation in different solvent systems, especially buffer solutions, in comparison with commercially available acetylthiomethyl(diphenyl)phosphine borane **16** to demonstrate its potential applicability in chemical peptide modifications.[10] For this purpose, we first wanted to probe the reactivity of **15** on a small water-soluble molecule. Therefore, we synthesized benzyl azide **17** bearing a short polyethylene glycol chain in the *meta* position to enhance its water solubility (Scheme 3, for synthesis of **17** see Scheme 5 in Supplementary data).



Scheme 2. Synthesis of phosphinothioester **15**.



Scheme 3. Traceless Staudinger ligation with benzyl azide **17** after acidic borane deprotection.

In order to activate water-soluble **15** for traceless Staudinger reactions, the borane protecting groups have to be removed first. In principle, both acidic and basic deprotection strategies can be envisioned, in which the latter one commonly proceeds best with 4 equiv DABCO in organic solvents like toluene or DMSO at 40 °C.[11,12,14–16] We decided to apply acidic deprotection conditions (e.g., TFA) for their better compatibility with the planned use of aqueous solvent systems. As shown before by our group, the acidic deprotection strategy has several advantages:[16b,21] the reaction is fast, proceeds at room temperature and TFA and borane are easily removable by evaporation under vacuum for subsequent solvent exchange to water. In addition, the acidic conditions do not yield the oxophilic phosphine that undergoes the traceless Staudinger ligation, but rather its protonated phosphonium salt. Thus, the stability towards air is enhanced and the reaction can be initiated by addition of base and change of pH after addition of all reagents.[22]

During these studies it became evident that diphenylphosphinomethanethiol **16** gave good results not only in organic solvents (54% conversion in pure DMF, Table 1, entry 1), but also in mixed aqueous solutions (73% conversion in 1:1 DMF/buffer, 0.4 M phosphate buffer, pH 8, Table 1, entry 7). Furthermore, in all transformations less than 10% P,N-bond hydrolysis to **19** were observed. With further increase of the water content, the efficiency of the ligation process with phosphine **16** dropped drastically. In contrast, the dimethylamine substituted derivative **15** reacted with azide **17** to full conversion in a 1:4 mixture of DMF and buffer.

Table 1
Conditions for traceless Staudinger ligation of phosphines **15** and **16** with benzyl azide **17**

Entry	Phosphine–borane	Solvent system	Ratio[a] (%)		
			17	**18**	**19**
1	**16**	DMF + DIPEA (12 equiv)	39	54	7
2	**15**	DMF + DIPEA (12 equiv)	54	39	7
3	**16**	Phosphate buffer (0.4 M, pH 8)	50	44	6
4	**15**	Phosphate buffer (0.4 M, pH 8)	74	21	5
5	**16**	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 4:1)	69	31	0
6	**15**	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 4:1)	0	88	12
7	**16**	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:1)	23	73	4
8	**15**	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:1)	47	45	8

[a] Determined by LC–UV at 280 nm (normalised to 100%). For more information, see the SI.

Besides minor formation of some amine **19** by P,N-bond hydrolysis, the sole product detected was the desired acetamide (Scheme 3). It is also important to note that increasing the amount of the buffer in the solvent system caused partial phosphine oxidation while adjusting the pH. Therefore, it became necessary to work under complete oxygen exclusion.

2.3. Traceless Staudinger ligation on azido peptides

These positive results encouraged us to investigate the reactivity of our compounds with azido peptide with unprotected side chains. We decided to probe the efficacy of both borane protected phosphino thioesters **15** and **16** in the reaction with an Fmoc-protected peptide **20** containing ε-azido-Lys in its structure (Scheme 4, Table 2). The N-terminal Fmoc group was kept for better UV detection and comparison of the peptidic compounds by LC–MS. To our knowledge, side chain acetylation of an azido norleucine peptide via the traceless Staudinger ligation has not been presented in the literature.

The peptide was synthesized manually by SPPS following the standard Fmoc protocol. All applied conditions for the traceless Staudinger ligation with diphenylphosphine reagent **16** failed. In every case we observed only traces of product **21** or no reaction occurred at all. Due to the peptide's hydrophobicity and therefore poor solubility in aqueous systems, conversions in buffer were also very slow for phosphine **15**. Upon addition of DMF, the azido peptide **20** started to dissolve and the reaction with phosphine **15** proceeded faster. The best conversion could be detected after 20 h in a 4:1 mixture of DMF and buffer. With only 3 equiv of phosphine we observed 42% (Table 2, entry 6) of the desired acetylated peptide. We can deduce from the amount of undesired amine **22** formed during the reaction (29%, entry 6) that rearrangement of the aza-ylide seems slower in the case of the peptide. Protonation of the nitrogen might proceed faster, leading to the hydrolysis of the aza-ylide. This could be due to the higher steric demand of the employed azido peptide **20** and the bulky Fmoc-group in close proximity. More test reactions with peptides not bearing the Fmoc group on the N-terminus might be necessary to get more information. It is noteworthy that a two-fold concentration of peptide **20** (Table 2, entry 11) did not result in any improvement in conversion and amide-to-amine ratio. The amount of observed amine side-product was even higher. In contrast and as expected, conversion could be increased by addition of more phosphine. With 10 equiv of phosphine **15** the product was formed in 61% with 33% of the undesired amine **22** (Table 2, entry 10).

3. Conclusion

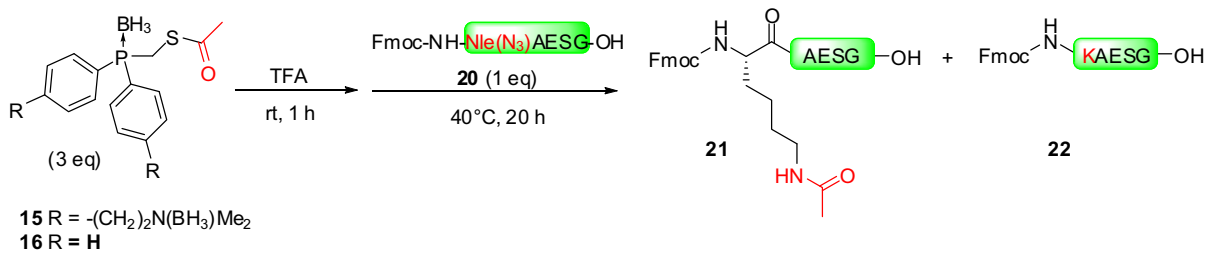
In summary, we presented an improved synthetic protocol for bis{4-[(*N*,*N*-dimethylaminoethyl)phenyl]}(acetylthiomethyl)phosphine tris(borane) (**15**). In addition, we compared this water soluble phosphine borane with commercially available diphenyl(acetylthiomethyl) phosphine borane (**16**) in selective acetylation reactions via traceless Staudinger ligation with two different azido compounds, both a water-soluble benzylic azide **17** and a more complex ε-azido norleucine containing peptide **20**. Compound **16** showed superior reactivity under oxygen-free reaction conditions in the acetyl transfer reaction with benzyl azide derivative **17** in a mixture of buffer and DMF (1:1); but with increasing amounts of buffer (80%) only phosphine **15** could yield an even higher product formation. For peptide **20**, only water soluble phosphine borane **15** was able to mediate the traceless Staudinger ligation for selective lysine side chain acetylation. The presented results show that water soluble phosphine boranes could have broad utility as a ligation reagent for selective peptide transformations as long as an oxygen-free environment can be ensured.

4. Experimental section

4.1. Materials and methods

4.1.1. Solvents and chemicals

All reactions were performed under argon atmosphere with dry and degassed solvents. All reagents were purchased from commercial suppliers and used without further purification. MeCN, DMF were dried over molecular sieves or purchased dry from ACROS.



Scheme 4. Traceless Staudinger ligation with azido peptide **20**.

Table 2
Conditions for traceless Staudinger ligation of phosphines **15** and **16** with Fmoc-protected azidopeptide **20**

Entry	Phosphine–borane (equiv)	*c* (peptide) (mM)	Solvent system	Ratio[a] (%)		
				20	**21**	**22**
1	**16** (3)	2	DMF + DIPEA (12 equiv)	100	0	0
2	**15** (3)	2	DMF + DIPEA (12 equiv)	86	12	2
3	**16** (3)	2	Phosphate buffer (0.4 M, pH 8)	80	17	3
4	**15** (3)	2	Phosphate buffer (0.4 M, pH 8)	98	2	0
5	**16** (3)	2	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:4)	91	7	1
6	**15** (3)	2	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:4)	28	42	29
7	**16** (3)	2	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 9:1)	95	5	0
8	**15** (3)	2	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:1)	88	11	1
9	**15** (5)	2	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:4)	7	44	49
10	**15** (10)	2	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:4)	6	61	33
11	**15** (3)	4	Phosphate buffer (0.4 M, pH 8)/DMF (v:v 1:4)	39	27	33

[a] Determined by LC–UV at 301 nm (normalised to 100%). For more information, see the SI.

THF, diethyl ether were dried over sodium/benzophenone ketyl and DCM was dried over P_2O_5. The resins as well as Fmoc-protected natural Lamino acids were purchased from Novabiochem. Thin-layer chromatography (TLC) was performed with precoated silica gel plates and visualized by UV light (λ = 254 nm), $KMnO_4$ solution or iodine on silica gel. The reaction mixtures were purified by column chromatography over silica gel (60–240 mesh).

4.1.2. Analytical methods

The NMR spectra were recorded on 500, 400 or 300 MHz spectrometers in $CDCl_3$ as a solvent at room temperature. Chemical shifts (δ) are reported in ppm relative to residual solvent peak ($CDCl_3$; 7.26 ppm for ^{1}H NMR and 77 ppm for ^{13}C NMR). Mass spectra were recorded on Shimazu GC–MS QP2010S spectrometer working in electron ionization (EI) mode using a Phenomenex Zebron ZB-35HT INFERNO column with the following parameters: pressure, 65 kPa; total flow, 33.9 mL/min; column flow, 1.0 mL/min; linear velocity, 36.8 cm/s; split 30; 35 min or 65 kPa; total flow, 23.9 mL/min; column flow, 1.0 mL/min; linear velocity, 36.8 cm/s; split 20; 15 min in presence of internal standard hexadecane. The analytical HPLC was applied from WatersTM with a 717 plus autosampler, a 600S controller, 2 pumps 616 and a 2489 UV/Visible detector connected to a 3100 mass detector. The RP-HPLC-column was a Kromasil C18 (5 μm, 250 × 4.6 mm with a flow rate of 0.8 mL/min, MeCN/H_2O (0.1% TFA)). Elementary analysis was performed on PERKIN ELMER CHN 2400.

4.1.3. Preparative HPLC

HPLC purification of the peptide was performed on a JASCO LC-2000 Plus system using a reversed phase C18 column (5 mL, 25 × 250 mm, constant flow of 16.0 mL/min: 5 min at 7% MeCN (with 0.1% TFA), gradient 7–95% MeCN (with 0.1% TFA) over 30 min (Gradient B)), consisting of a Smartline Manager 5000 with interface module, two Smartline Pump 1000 HPLC pumps, a 6-port-3-channel injection valve with 2.5 mL loop, a UV detector (UV-2077) and a high pressure gradient mixer.

4.1.4. Peptide synthesis

Peptides were synthesised with ABI 433A Peptide Synthesizer from Applied Biosystems via standard Fmoc-based conditions (Fast-moc protocol with HOBt/HBTU conditions) on a preloaded Wang resin (0.79 mmol/g).

4.2. General synthesis

4.2.1. Synthesis of 2-(4-bromophenyl)ethanol 10

The solution of 1,4-dibromobenzene (40 g, 0.17 mol) in dry diethyl ether (200 mL) was cooled to −40 °C and *n*-buthyllithium (105.6 mL, 1.6 M in hexanes, 0.17 mol) was added dropwise for 1 h. The mixture was allowed to warm up to rt and stirred for 2 h. The reaction was once again cooled to −40 °C and a solution of ethylene epoxide (8.46 mL, 0.17 mol) in 40 mL of diethyl ether was added slowly to the mixture. The reaction mixture was slowly warmed to room temperature and stirred for 1 h at room temperature. Then 40 mL of NH_4Cl was added to quench the reaction. The product was extracted with diethyl ether (3 × 100 mL). The organic layers were dried over $MgSO_4$, filtered and concentrated. Distillation afforded 28.63 g (85%) of a colourless liquid of **10**. Bp 120–127 °C (1 mmHg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (br s, 1H); 2.81 (t, J = 6.42 Hz, 2H); 3.83 (t, J = 6.60 Hz, 2H); 7.09–7.11 (m, 2H); 7.42–7.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.5; 63.3; 120.3; 130.7; 131.6; 137; GC t_R = 6.406 min (s), 7 min (broad peak); GC–MS (EI, 70 eV) *m*/*z* (%) 202 [M^+] (24), 200 (25), 172 (22), 171 (71), 170 (24), 169 (71), 121 (15), 92 (9), 91 (100), 90 (63), 89 (53). For GC–MS results see SI (Figs. S1 and S2).

4.2.2. Synthesis of 4-bromophenethyl methanesulfonate 11

To a solution of **10** (28.63 g, 0.142 mol) in 300 mL of CH_2Cl_2 was added triethylamine (29.5 mL, 0.21 mol), the mixture was cooled to 0 °C and mesyl chloride (15.4 mL, 0.2 mol) was added dropwise. The reaction was stirred for 48 h at room temperature. Then 50 mL of a 0.1 N HCl solution was added and the mixture was extracted with CH_2Cl_2 (3 × 150 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude solid was crystallized from a mixture of CH_2Cl_2 and hexane. The pure compound **11** was obtained as a pale white solid (38.3 g, 96%). Mp 58–60 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (s, 3H); 3.02 (t, J = 6.78 Hz, 2H); 4.40 (t, J = 6.97 Hz, 2H); 7.11–7.14 (m, 2H); 7.45–7.47 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.0; 37.4; 63.6; 121.0; 130.7; 131.8; 137.3; GC t_R = 15.2 min; GC–MS (EI, 70 eV) *m*/*z* 280 [M^+] (0.1), 185 (11), 184 (98), 183 (12), 182 (100), 171(37), 169 (37), 104 (24), 103 (38), 91 (9), 90 (42), 89 (34); Anal. Calcd for $C_9H_{11}BrO_3S$: C, 38.72; H, 3.97. Found: C, 38.9; H, 3.88. For GC–MS results see SI (Figs. S3 and S4).

4.2.3. Synthesis of 2-(4-bromophenyl)-*N*,*N*-dimethylethylamine 12

To a solution of mesylate **11** (38.3 g, 0.138 mol) in 200 mL of dry THF dimethylamine (274 mL, 2 M in THF, 0.549 mol) was added. The mixture was stirred at 45 °C for 24 h and then allowed to reach room temperature. Dimethylamine (137 mL, 2 M in THF, 0.275 mol) was once again added and the resulting solution was heated for another day at 45 °C. The precipitate was filtered off and the filtrate was concentrated. The resulting residue was purified by flash column chromatography (chloroform/methanol, 15:1) to give **12** as an orange oil 28.77 g (97%). R_f = 0.6 (chloroform/methanol, 15:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 6H); 2.50–2.52 (m, 2H); 2.71–2.75 (m, 2H); 7.07–7.09 (m, 2H); 7.39–7.41 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.7; 45.4; 61.2; 119.7; 130.3; 131.4; 139.3; GC t_R = 9.5 min; GC–MS (EI, 70 eV) *m*/*z* 228 [M^+] (3), 226 (5), 171 (16), 169 (17), 104 (64), 103 (54), 102 (27), 91 (21), 90 (100), 89 (81). For GC–MS results see SI (Figs. S5 and S6).

4.2.4. Synthesis of bis{4-[(*N*,*N*-dimethylamino)ethyl]phenyl}(acetylthiomethyl)phosphine oxide 13

In a 500 mL flame-dried three-necked flask equipped with a reflux condenser magnesium turnings (1.88 g, 0.077 mol) were placed under argon and 80 mL of anhydrous THF was added, followed by **12** (15.05 g, 0.066 mol) and a catalytic amount of I_2.

The mixture was refluxed for 3 h until most of the magnesium was consumed. The Grignard reagent partially precipitated. The resulting mixture was allowed to cool to room temperature and 50 mL of anhydrous THF were added. The reaction mixture was cooled to 0 °C and diethyl phosphite (2.55 mL, 0.019 mol) was added. The mixture was stirred for 24 h at room temperature. Then the reaction mixture was cooled to 0 °C and was quenched with 10 mL of brine and THF was evaporated. The residue was extracted with CH_2Cl_2 (5 × 150 mL), dried over $MgSO_4$, filtered and concentrated. The crude material was washed by flash column chromatography (chloroform/methanol + 0.5% Et_3N, 15:1) to elute less polar impurities and then the product was eluted with chloroform/methanol (4:1 + 0.5% Et_3N) to obtain a pale yellow thick oil (4.55 g, 67%). R_f = 0.25 (chloroform/methanol 4:1). ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 12H); 2.55–2.57 (m, 4H); 2.81–2.85 (m, 4H); 7.31–7.4 (m, 4H); 7.57–7.63 (m, 4H); 8.04 (d, J_{P-H} = 468.66 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.1 (d, J_{P-C} = 1.2 Hz); 45.2; 60.7; 128.4 (d, J_{P-C} = 16.7 Hz); 129.2 (d, J_{P-C} = 13.2 Hz); 129.5 (d, J_{P-C} = 24.1 Hz); 130.7; 130.9; 145.3 (d, J_{P-C} = 2.9 Hz); ^{31}P (121 MHz, $CDCl_3$) δ 21.67 ppm (s).

4.2.5. Synthesis of bis{4-[(*N*,*N*-dimethylamino)ethyl]phenyl} phosphine tris(borane) 14

The solution of secondary phosphine oxide **13** (450 mg, 1.28 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise to a solution of DIBAl-H (1.5 M in toluene, 4.26 mL, 6.4 mmol) under argon in a 100 mL flame-dried three-neck flask. The resulting solution was stirred for 1 h, then cooled to 0 °C. The solution was diluted with anhydrous CH_2Cl_2 (20 mL) and argon was bubbled through the solution for 10 min. A solution of 2 N NaOH (5 mL) was added dropwise to the reaction mixture, followed by brine (5 mL). The resulting mixture was transferred to an extraction funnel and the organic layer was separated, dried over $MgSO_4$, filtered under an inert atmosphere and concentrated under reduced pressure to 10 mL. The resulting solution was cooled to 0 °C with an ice bath under Ar and a borane dimethyl sulfide complex (0.82 mL, 8.69 mmol) was added drop wise. The resulting reaction mixture was allowed to warm slowly to room temperature overnight. The solvent was removed under reduced pressure, and the crude oil was purified by flash column chromatography (silica gel, CH_2Cl_2) to give phosphine–borane complex **14** as a white solid in 49% yield (0.24 g). ^1H NMR (500 MHz, $CDCl_3$) δ 0.79–1.95 (br m, 9H), 2.66 (s, 12H), 2.93–2.95 (m, 4H), 3.10–3.13 (m, 4H), 6.27 (dq, J_{P-H} = 374 Hz, 1H), 7.30–7.33 (m, 4H), 7.60–7.62 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.9, 51.9; 65.7; 127.0, 129.6 (d, J_{P-C} = 9.99 Hz); 133.4 (d, J_{P-C} = 10.0 Hz); 142.2 (d, J_{P-C} = 2.7 Hz); ^{31}P NMR (202 MHz, $CDCl_3$) δ −0.12 ppm (m). Anal. Calcd for $C_{20}H_{38}B_3N_2P$: C, 70.18; H, 9.42; N, 8.18. Found: C, 70.1; H, 9.23; N, 7.97.

4.2.6. Synthesis of bis{4-[(*N*,*N*-dimethylamino)ethyl]phenyl} (acetylthiomethyl)phosphine tris(borane) 15

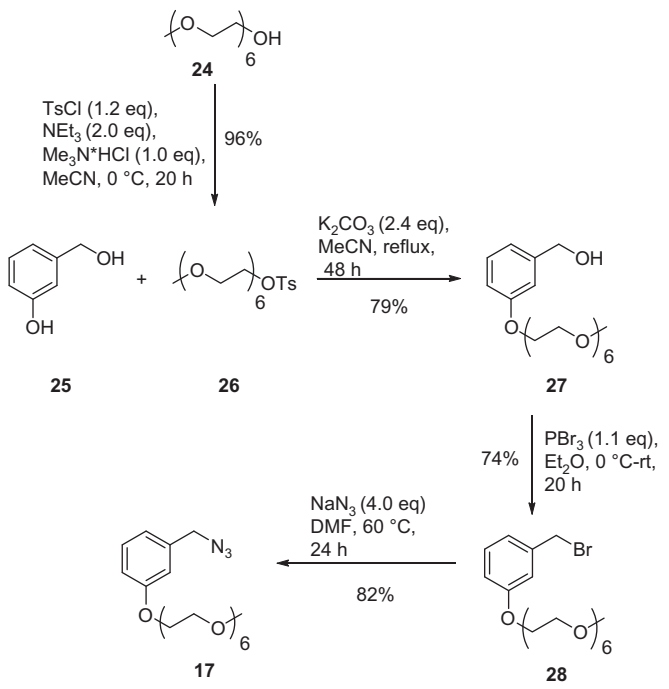
Secondary phosphine–borane **14** (200 mg, 0.54 mmol) was dissolved in dry DMF (2 mL) under argon and cooled to 0 °C. NaH (22 mg, 0.54 mmol) was added and the mixture was stirred at 0 °C until bubbling stopped. Acetylthiomethyl bromide (110 mg, 0.65 mmol) was added and the resulting mixture was allowed to warm to room temperature and stirred for 14 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (2% v/v ethyl acetate and 28% hexanes in CH_2Cl_2) to give phosphine–borane **15** as a colourless oil in 60% yield. ^1H NMR (500 MHz, $CDCl_3$) δ 2.26 (s, 3H), 2.66 (s, 12H), 2.94–2.96 (m, 4H), 3.10–3.13 (m, 4H), 3.76 (d, J_{P-H} = 6.94 Hz, 2H), 7.29–7.31 (m, 4H), 7.60–7.64 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.5, 23.8, 30.1, 31.0, 51.9, 65.4 (d, J_{P-C} = 34.0 Hz), 125.8 (d, J_{P-C} = 56.8 Hz), 126.9, 127.6, 128.5, 129.3 (d, J_{P-C} = 10.2 Hz), 132.8 (d, J_{P-C} = 10 Hz), 142.3 (d, J_{P-C} = 2.5 Hz), 193.1 (d, J_{P-C} = 3.0 Hz). ^{31}P NMR (202 MHz, $CDCl_3$): δ = 18.07 ppm (m). HRMS (ESI-ToF): m/z = 445.2758 $[M-BH_3+H]^+$ (calcd: m/z = 445.2780) (Scheme 5).

4.2.7. Synthesis of tosylated hexaethylenglycol-monomethylether 26

Hexaetheleneglycol monomethyl ether **24** (1 mL, 3.64 mmol) was dissolved in dry acetonitrile (200 mL) and triethylamine (1.01 mL, 7.29 mmol) was added. The mixture was cooled to 0 °C. Solid *p*-toluenesulfonyl chloride (0.834 g, 4.37 mmol) was added as well as trimethylamine hydrochloride (0.348 g, 3.64 mmol). The resulting reaction mixture was stirred overnight at 0 °C. Then the reaction mixture was allowed to warm to room temperature and acetonitrile was evaporated. The residue was dissolved in 100 mL of CH_2Cl_2 and 50 mL of 1 M hydrochloric acid were added. The mixture was extracted with CH_2Cl_2 (70 mL × 5). The combined organic fractions were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The obtained product **26** was used without any further purification (1.588 g, 96%). ^1H NMR (500 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.36 (s, 3H), 3.52–3.54 (m, 2H), 3.56 (br s, 3H), 3.58–3.61 (m, 5H), 3.62–3.64 (m, 10H), 3.65–3.68 (m, 2H), 4.13–4.15 (m, 2H), 7.32–7.33 (m, 2H), 7.77–7.79 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.6, 59.0, 68.6, 69.2, 70.4, 70.5, 70.5, 70.7, 71.9, 127.9, 129.8, 132.9, 144.7.

4.2.8. Synthesis of 3-(2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)ethoxy)ethoxy)ethoxy)benzyl alcohol 27

To a solution of tosylated hexaethylenglycol–monomethylether **26** (1.588 g, 3.55 mmol) and potassium carbonate (1.18 g, 8.53 mmol) in dry acetonitrile (40 mL), 3-hydroxybenzyl alcohol **25** (0.485 g, 3.9 mmol) was added. The resulting reaction mixture was stirred for 48 h at reflux. Then, the reaction mixture was allowed to reach room temperature and the solution was filtered and concentrated. The residue was purified by column chromatography (ethyl acetate/methanol, 10:1) to obtain a yellowish oil (0.936 g, 79%). ^1H NMR (500 MHz, $CDCl_3$) δ 3.36 (s, 3H), 3.52–



Scheme 5. Synthesis of benzyl azide **17**.

3.54 (m, 2H), 3.61–3.64 (m, 13H), 3.65–3.67 (m, 3H), 3.70–3.72 (m, 2H), 3.83–3.85 (m, 2H), 4.12–4.14 (m, 2H), 4.64 (d, $J_{H\text{-}H}$ = 3.6 Hz, 2H), 6.81–6.84 (m, 1H), 6.91–6.96 (m, 2H), 7.22–7.25 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.0, 65.1, 67.83, 69.7, 70.4, 70.5, 70.5, 70.6, 70.6, 70.8, 71.9, 113.0, 113.8, 119.2, 129.5, 142.7, 159.0; HRMS (ESI-ToF): m/z = 403.1943 $[M+H]^+$ (calcd: m/z = 403.2326).

4.2.9. Synthesis of 3-(2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)ethoxy)ethoxy)ethoxy)benzyl bromide 28

To a solution of PEG-functionalized benzyl alcohol **27** (0.6 g, 15 mmol) in dry diethyl ether (50 mL) phosphorus tribromide (0.16 mL, 16.4 mmol) was added. The resulting reaction mixture was stirred for 2 h at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. Then the reaction mixture was poured into 50 mL of ice water and extracted with diethyl ether (50 mL × 5). The combined organic fractions were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure giving a yellow oil. Compound **28** was used without any further purification (0.51 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.37 (s, 3H), 3.53–3.55 (m, 2H), 3.62–3.66 (m, 14H), 3.67–3.68 (m, 2H), 3.71–3.73 (m, 2H), 3.84–3.86 (m, 2H), 4.12–4.14 (m, 2H), 4.45 (s, 2H), 6.83–6.85 (m, 1H), 6.94–6.97 (m, 2H), 7.21–7.24 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.0, 67.430, 69.5, 70.5, 70.5, 70.5, 70.6, 70.8, 71.9, 114.7, 115.2, 121.4, 129.7, 139.1, 158.9.

4.2.10. Synthesis of 3-(2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)ethoxy)ethoxy)ethoxy)benzyl azide 17

Sodium azide (0.278 g, 4.3 mmol) was added to a solution of benzyl bromide **28** in dry DMF (6 mL). The reaction was stirred for 24 h at 60 °C. The reaction mixture was allowed to reach room temperature and the solvent was evaporated. 20 mL of brine were added to the resulting residue and the product extracted with diethyl ether (5 × 50 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated. Desired azide **17** was obtained as a pale oil (0.374 g, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 (s, 3H), 3.52–3.55 (m, 2H), 3.62–3.66 (m, 14H), 3.67–3.68 (m, 2H), 3.71–3.73 (m, 2H), 3.84–3.86 (m, 2H), 4.12–4.14 (m, 2H), 4.29 (s, 2H), 6.87–6.89 (m, 3H), 7.25–7.29 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 54.7, 59.0, 67.4, 69.6, 70.5, 70.5, 70.6, 70.6, 70.8, 71.9, 114.4, 114.4, 120.5, 129.8, 136.8, 159.1; HRMS (ESI-ToF): m/z = 428.2080 $[M+H]^+$ (calcd: m/z = 428.2391).

4.2.11. General procedure of acidic deprotection of 15 or 16

Borane-protected phosphine **15** or **16** (3 μmol) was placed in an eppendorf tube (0.5 mL) under argon and treated with 0.15 mL of 99% TFA. The mixture was placed in a shaker for 1 h at room temperature. Then TFA was evaporated under high vacuum for 1 h.

4.2.12. Procedure of application of 15 or 16 in traceless Staudinger ligation with 17 in DMF

Borane-protected phosphine **15** or **16** (3 μmol) was deprotected according to the general procedure. To a solution of the residual phosphonium salt of **15** or **16** in DMF (50 μL) was added azide **17** (1.283 mg, 3 μmol), DIPEA (6.29 μL, 36 μmol). The mixture was heated at 40 °C for 24 h and then the ratio of azide **17**, acetamide 1**8** and amine **19** was determined by LC–UV (0% MeCN (0.1% TFA) for 5 min, 0–100% MeCN (0.1%TFA) in 35 min, rt: **17**: 28.010 min, **18**: 21.542 min, **19**: 18.730 min). The different molecule peaks were assigned by LRMS (ESI-ToF): m/z (azide **17**) = 450.2 $[M+Na]^+$ (calcd: m/z = 450.2), m/z (acetamide **18**) = 444.0 $[M+H]^+$ (calcd: m/z = 444.3), m/z (amine **19**) = 402.2 $[M+H]^+$ (calcd: m/z = 402.2). For LC–UV spectra see SI (Figs. S7 and S8).

4.2.13. Procedure of application of 15 or 16 in traceless Staudinger ligation with 17 in mixed solvent systems or pure buffer (0.4 M phosphate buffer, pH 8)

Borane-protected phosphine **15** or **16** (3 μmol) was deprotected according to the general procedure. To the residual phosphonium salt of **15** or **16** were added 50 μL solvent (Table 1, entries 3–8) and azide **17** (1.283 mg, 3 μmol) under argon. The pH was adjusted to 8.0. The mixture was placed in a shaker and heated at 40 °C for 24 h. The ratio of azide **17**, actamide 1**8** and amine **19** was determined by LC–UV (0% MeCN (0.1% TFA) for 5 min, 0–100% MeCN (0.1%TFA) in 35 min, rt: **17**: 28.010 min, **18**: 21.542 min, **19**: 18.730 min). The different molecule peaks were assigned by LRMS (ESI-ToF): m/z (azide **17**) = 450.2 $[M+Na]^+$ (calcd: m/z = 450.2), m/z (acetamide **18**) = 444.0 $[M+H]^+$ (calcd: m/z = 444.3), m/z (amine **19**) = 402.2 $[M+H]^+$ (calcd: m/z = 402.2). For LC–UV spectra see SI (Figs. S9–S16).

4.2.14. Procedure for synthesis of azido peptide 20

The peptide was synthesized manually by standard coupling conditions following the Fmoc-protocol on a preloaded Fmoc-Gly-Wang resin (0.79 mmol/g; 0.10 mmol)) The natural amino acids (10 equiv) were coupled with HBTU, HOBt and DIPEA in DMF for 1.5 h. *N*-Fmoc-6-azidonorleucine (2 equiv) was coupled with HATU, HOBt and DIPEA in DMF for 6 h at rt. Purification by preparative LCMS yielded the desired peptide **20** (27.90 mg, 38%): HRMS: m/z (ESI-ToF) $[M+H]^+$ 739.3016, m/z (calcd) $[M+H]^+$ 739.3046.

4.2.15. Procedure of application of 15 or 16 in traceless Staudinger ligation with 20 in DMF

Borane-protected phosphine **15** or **16** (3 μmol) was deprotected according to the general procedure. To a solution of the residual phosphonium salt of **15** or **16** in DMF (250 μL) under argon was added azido peptide **20** (370 μg, 0.5 μmol) and DIPEA (1.1 μL, 6.01 μmol). The mixture was heated at 40 °C for 24 h and the ratio of azide **20**, actamide **21** and amine **22** was determined by LC–UV (0% MeCN (0.1% TFA) for 5 min, 0–100% MeCN (0.1%TFA) in 35 min, rt: **20**: 24.726 min, **21**: 22.436 min, **22**: 21.463 min). The different molecule peaks were assigned by LRMS (ESI-ToF): m/z (azide **20**) = 739.2 $[M+H]^+$ (calcd: m/z = 739.2), m/z (acetamide **21**) = 755.2 $[M+H]^+$ (calcd: m/z = 755.3), m/z (amine **22**) = 713.2 $[M+H]^+$ (calcd: m/z = 713.3). For LC–UV spectra see SI (Figs. S17 and S18).

4.2.16. Procedure of application of 15 or 16 in traceless Staudinger ligation with 20 in mixed solvent systems or pure buffer (0.4 M phosphate buffer, pH 8)

Borane-protected phosphine **15** or **16** (3 μmol Table 2, entries 3–8; 5 μmol entry 9; 10 μmol entry 10) was deprotected according to the general procedure. To the residual phosphonium salt of **15** or **16** were added 250 μL solvent (Table 2, entries 3–11) and azido peptide **20** (370 μg, 0.5 μmol) under argon. The pH was adjusted to value 8. The mixture was placed in a shaker and heated at 40 °C for 24 h. The ratio of azide **20**, actamide **21** and amine **22** was determined by LC–UV (0% MeCN (0.1% TFA) for 5 min, 0–100% MeCN (0.1% TFA) in 35 min, rt: **20**: 24.726 min, **21**: 22.436 min, **22**: 21.463 min). The different molecule peaks were assigned by LRMS (ESI-ToF): m/z (azide **20**) = 739.2 $[M+H]^+$ (calcd: m/z = 739.2), m/z (acetamide **21**) = 755.2 $[M+H]^+$ (calcd: m/z = 755.3), m/z (amine **22**) = 713.2 $[M+H]^+$ (calcd: m/z = 713.3). For LC–UV spectra see SI (Figs. S19–S26).

4.2.17. Application of 15 in traceless Staudinger ligation with 20 at 4 mM concentration of azidopeptide 20 (Table 2, entry 11)

Borane-protected phosphine **15** or **16** (3 μmol) was deprotected according to the general procedure. To the residual phosphonium

salt of **15** or **16** were added 250 μL of a mixture of DMF and the buffer (4:1) and azido peptide **20** (738 μg, 1.0 μmol) under argon. The pH was adjusted to value 8. The mixture was placed in a shaker and heated at 40 °C for 24 h. The ratio of azide **20**, actamide **21** and amine **22** was determined by LC–UV (0% MeCN (0.1% TFA) for 5 min, 0–100% MeCN (0.1% TFA) in 35 min, rt: **20**: 24.726 min, **21**: 22.436 min, **22**: 21.463 min). The different molecule peaks were assigned by LRMS (ESI-ToF): *m*/*z* (azide **20**) = 739.2 $[M+H]^+$ (calcd: *m*/*z* = 739.2), *m*/*z* (acetamide **21**) = 755.2 $[M+H]^+$ (calcd: *m*/*z* = 755.3), *m*/*z* (amine **22**) = 713.2 $[M+H]^+$ (calcd: *m*/*z* = 713.3). For LC–UV spectra see SI (Fig. S27).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.02.045.

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22. In addition, the acidic deprotection strategy allows also the global deprotection of phosphino thioester peptides that can be applied in chemoselective peptide cyclizations as shown by our group.[16b]