A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition

GDCh

Chemie

www.angewandte.org

Accepted Article

Title: Efficient Pictet Spengler Bioconjugation with N-Substituted Pyrrolyl Alanine Derivatives

Authors: Sebastian Pomplun, Mohamed YH Mohamed, Tobias Oelschlaegel, Christian Wellner, and Frank Bergmann

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201814200
Angew. Chem. 10.1002/ange.201814200

Link to VoR: http://dx.doi.org/10.1002/anie.201814200
http://dx.doi.org/10.1002/ange.201814200

WILEY-VCH

10.1002/anie.201814200

WILEY-VCH

COMMUNICATION

Efficient Pictet Spengler Bioconjugation with *N*-Substituted Pyrrolyl Alanine Derivatives

Sebastian Pomplun,*[a] Mohamed YH Mohamed, [a] Tobias Oelschlaegel, [a] Christian Wellner, [a] Frank Bergmann[a]

Abstract: *N*-pyrrolyl alanine derivatives are described here as efficient reagents for the fast and selective Pictet Spengler reaction with aldehyde containing biomolecules. Other aldehyde labeling methods described so far have several drawbacks, like hydrolytic instability, slow reaction kinetics or not readily available labeling reagents. The reaction speed in Pictet Spengler cyclizations of pyrrolyl 2-ethylamine substituted at the pyrrole nitrogen is shown to be significantly faster than the analogues substituted at the α- and β- position and functionalized *N*-pyrrolyl alanine derivatives can be easily synthesized in only 2-3 steps from commercially available materials. The small size of the reagent, the fast reaction rate and the easy synthesis make pyrrolyl alanine Pictet Spengler (PAPS) an attractive choice for bioconjugation reactions. PAPS was shown as an efficient strategy for the site selective biotinylation of an antibody as well as for the condensation of nucleic acid derivatives, proofing the versatility and usefulness of this novel reagent.

Bioconjugation reactions are essential tools in modern chemical biology.[1] Those reactions enable the condensation of biomolecules, postsynthetic labeling of biomolecules with small molecules, immobilization of biomolecules on solid supports and more. In the field of protein conjugation early methodologies focused on modifications based on the reactivity of single amino acids (e.g N-hydroxysuccinimide (NHS) conjugation to lysine, or maleimide conjugation to cysteine), but in the last years site specific derivatizations have emerged as preferred strategy to obtain unambiguously predictable and defined conjugates.[2] Among several techniques available to date, the introduction of aldehydes into proteins has been shown as a versatile and profitable methodology to provide site-specifically a reactive moiety which can undergo chemoselective conjugation reactions with nucleophiles in an orthogonal manner to all other functional groups exposed by a protein. A variety of chemical, enzymatic, and genetic methods have been developed to introduce aldehydes into proteins site-specifically.[3] These include periodate oxidation of N-terminal serine or threonine residues,[4] pyridoxal phosphate-mediated N-terminal transamination to yield an alpha-ketoamide or glyoxamide,[5] genetic encoding of peptide tags that direct enzymatic ligation of aldehyde- or ketone-bearing small molecules[6] and genetic encoding of a site for modification by the formylglycine generating enzyme (FGE), the "aldehyde tag" method developed by Carrico and Bertozzi.[7] In the last years many techniques for labeling the protein via the aldehyde function have been described.[3, 8] Among these techniques there are for example the classical oxime or hydrazone ligation,[9] Pictet Spengler (PS) reaction[10] and the improved *iso*-Pictet Spengler type conjugation developed by Bertozzi and Rabuka[11]. The oxime ligation of aldehydes is limited by the hydrolytic instability of the formed conjugates. Oximes formed at α-oxo aldehydes seemed to be more resistant to hydrolysis,[12] but here the labeling position is constrained to the N-terminus of the protein, which could result as a severe limitation, e.g. when this causes impairment of antibody binding performance. In the Pictet Spengler based conjugation an irreversible covalent ring closure affords stable products. To overcome the extremely slow reaction kinetics of tryptamine based Pictet Spengler bioconjugation, a modified Pictet Spengler conjugation, where a hydrazine or hydroxylamine substituent is placed in the α-position of an indole, has been proposed [11]. The main limitation of this technique is the multistep synthesis of the alpha-hydrazino/hydroxylamine-indole building block (which makes this methodology inaccessible for laboratories without a strong synthesis background) and the relatively big size of the tricyclic conjugation moiety.

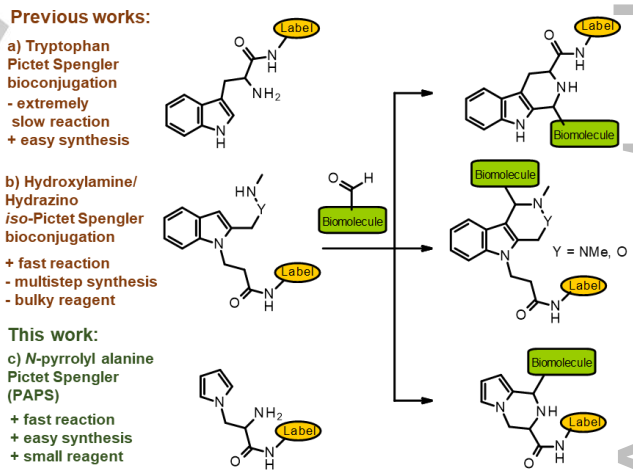


Figure 1. Bioconjugation reactions based on Pictet Spengler cyclization. a) Classic Pictet Spengler using a labeled tryptophan. This reaction is limited by extremely slow reaction kinetics. b) Hydroxylamine/hydrazino *iso*-Pictet Spengler: the reaction rates are significantly higher compared to tryptophan, while the reagent is still quite bulky and has to be prepared by a multistep synthesis. c) *N*-pyrrolyl alanine Pictet Spengler (PAPS), described in this work, enables fast bioconjuagtion, using a small and easy to synthesise reagent.

Since Pictet Spengler reaction of histamine with acetaldehyde under physiological conditions has been described[13] we envisioned this natural amino acid as a promising starting

[a] Dr. Sebastian Pomplun*+, Dr. Mohamed Yosry Mohamed, Dr. Tobias Oelschlaegel, Dr. Christian Wellner, Dr. Frank Bergmann
Roche Diagnostics GmbH
Nonnenwald 2, 82377 Penzberg
*E-mail: sebastian.pomplun@gmail.com
+Current address: Massachussetts Institute of Technology, Department of Chemistry, 77 Massachusetts Ave, Cambridge, MA 02139, USA

Supporting information for this article is given via a link at the end of the document.

10.1002/anie.201814200

point for the development of a smaller and synthetically more accessible bioconjugation reagent.
As test system containing an aldehyde, we synthesized formyl glycine containing peptide **1**[14] (Figure 2a), which was incubated with biotinylated histidine (**2**) for 24 h at 37 °C and pH = 5 and at pH 7.4 (conditions described by Ohya and Niitsu[13]). Since in both conditions only the semistable imine intermediate was observed (data not shown), while no Pictet Spengler ring closure occurred, we decided to analyze other histidine analogues for the possibility to undergo biocompatible Pictet Spengler reaction. In particular we envisioned to overcome the low reactivity of the electron poor imidazole heterocycle by using electron richer variants of this structure: 10 equivalents of each of histamine (**3**), 1-methyl imidazole (**4**), 3-methyl imidazole (**5**) and α–pyrrole 2-ethylamine (**6**) were added to peptide **1** and incubated for 24 h at 37 °C and pH = 5.4. While in the case of the histamine and the methylimidazoles no ring closure occurred, the pyrrolyl derivative (**6**) showed full conversion of the aldehyde into a stable Pictet Spengler product.

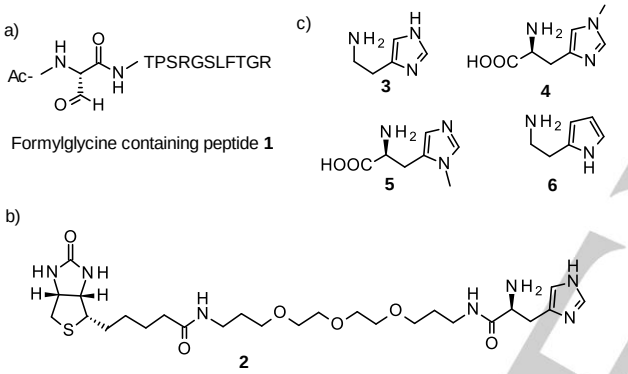


Figure 2. a) Formylglycine containing peptide (**1**). b) Biotinylated PEG-histidine (**2**). c) Histamine analogues (**3 – 6**).

Ongoing from this gratifying result we decided to examine other pyrrolyl-2-ethylamines regarding their reaction speed, since this has been shown as the major limitation of classical Pictet Spengler with tryptophan derivatives. While indoles show higher reactivity at their β-position in electrophilic substitution[11a], pyrroles preferentially react at their α–position, suggesting that the 2-ethylamine moiety should either be placed in the β-position of the pyrrole ring or at the nitrogen. To examine this hypothesis, α–, β– and *N*- 2-ethylamine substituted pyrroles (**6**,**7** and **8**) were reacted with peptide **1** and the conversion rates were observed by LCMS (Figure 3). The reaction was performed with 5 equivalents of pyrrole at a peptide concentration of 0.5 mM in acetate buffer at 37°C and pH 5.4. While surprisingly no significant rate difference between the α– and the β–2-ethylamine substituted pyrrole was observed, the *N*-substituted pyrrole displayed a significantly faster conversion rate, achieving a complete conversion of the formylglycine peptide **1** in the Pictet Spengler product (**11**) within 7 hours. We therefore chose this regioisomer for further investigations.
For straightforward installation of further moieties on the pyrrolyl *N*-ethylamine core, while keeping the reagent size as small as possible, we envisioned β-(*N*-pyrrolyl)-alanine (**14**, from here called *N*-pyrrolyl alanine to simplify the text) as ideal reagent. *N*-pyrrolyl alanine with either Fmoc or Boc protection on the amino functionality was obtained in only one step by Paal-Knorr pyrrole synthesis condensing dimethoxy

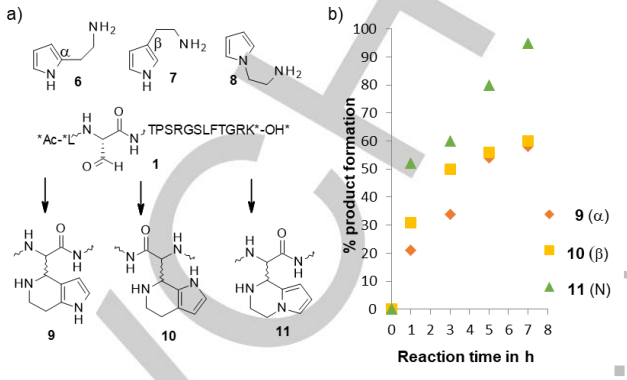


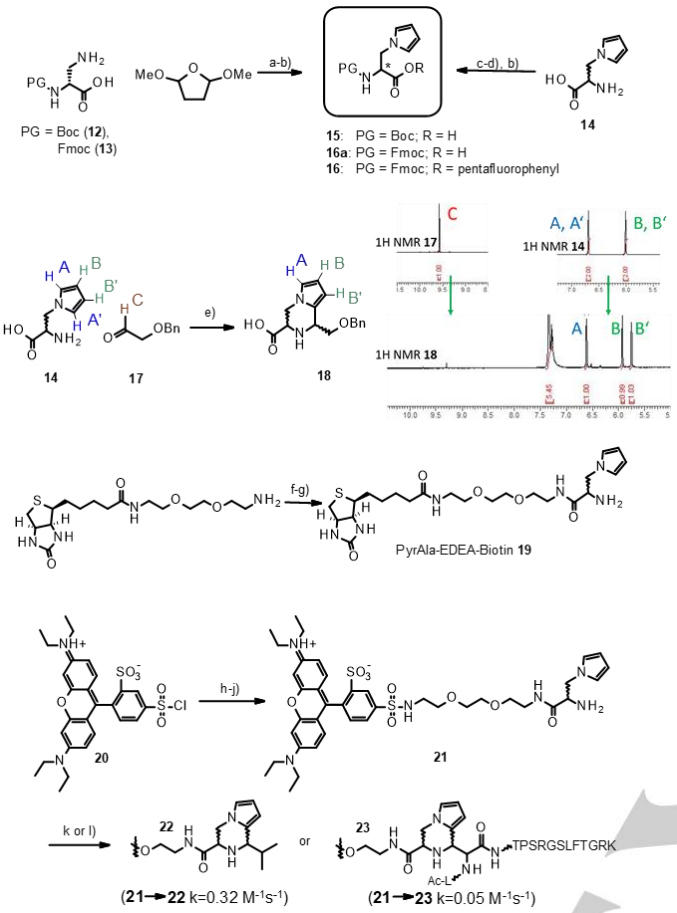
Figure 3. a) Conditions: 10 µL of peptide **1** (10 mM aq. sol.) and 20 µL of amine (50 mM aq. sol.) were added to 170 µL of acetate buffer (0.05 M, pH = 5.4), 37 °C. b) Conversion rates of the three pyrrolyl ethylamines. Reactions were followed by LCMS. After 24 h quantitative product formation was observed also for **9** and **10**.

tetrahydrofuran with Fmoc-NH-L-Dap-OH or Boc-NH-L-Dap-OH, respectively, under acidic conditions (Scheme 1). The Fmoc protected pyrrolyl alanine was further transformed into the active pentafluorophenyl ester (**16**), affording in overall only two steps a practical reagent to be coupled to any amino group in a label molecule of interest. Since unprotected pyrrolyl alanine (**14**) is also commercially available, derivatives **15** and **16** could be obtained by direct protection of this unnatural α–amino acid. Although both Paal-Knorr pyrrole synthesis and direct protection of **14** would allow chirality conservation of a defined stereocenter, at this point of the research we considered the stereocontrol of marginal importance in the context of bioconconjugation and we synthesized most of the following derivatives starting from stereochemically undefined **14**. To confirm accurately the formation of a covalent Pictet Spengler ring closure product, we condensed **14** with 2-(Benzyloxy)acetaldehyde (simply by dissolving the two substrates in a 1:1 mixture of MeCN and 0.1 M sodium acetate buffer at pH = 5 and removing the solvent after 1 h). The product NMR (Scheme 1, **18**) shows clearly the disappearing of both the aldehyde proton and of one of the pyrrole protons. The Pictet Spengler ring closure occurs with no stereocontrol, forming a second undefined stereocenter.
For the synthesis of a biotin labeled pyrrolyl alanine (**19**), suitable for detectable protein conjugation, **16** was coupled to biotin-EDEA (EDEA = 2,2'-(Ethylenedioxy)bis(ethylamine) and subsequently Fmoc deprotected. To enable the determination of the *N*-pyrrolyl alanine Pictet Spengler (PAPS) reaction rate by LCMS, we synthesized also a sulforhodamine-B-pyrrolyl alanine derivative (**21**).

10.1002/anie.201814200

WILEY-VCH

COMMUNICATION



Scheme 1. Reagents and conditions: a) CH_3COOH, 120 °C, 10 min, 24% (**15**), 53% (**16a**); b) Pentafluorophenol trifluoroacetate, DIPEA, CH_2Cl_2, 0°C, 1 h, 40 %; c) $NaHCO_3$, Boc_2O, 1,4-dioxane/H_2O, r.t., 16 h, quant.; d) Na_2CO_3, Fmoc-Cl, THF/H_2O, 0 °C – r.t., 4 h, 57 %; e) MeCN, acetate buffer, pH = 5, r.t., 1 h, quant. f) **16**, DIPEA, DMF, 45 min, r.t.; g) Et_2NH, 10 min, r.t.; h) EDEA, MeCN, 2 h, r.t., 83 %; i) 15, HATU, DIPEA, DMF, 20 min, r.t.; j) CF_3COOH + 5 % H_2O, 10 min, 28 %; k) Isobutyraldehyde, acetate buffer, pH = 5, r.t. l) **1**, acetate buffer, pH = 5, r.t.. DIPEA = N,N-Diisopropylethylamine; THF = tetrahydrofuran; DMF = dimethylformamide; EDEA = 2,2'-(Ethylenedioxy)bis(ethylamine).

For kinetic measurements **21** was conjugated to isobutyraldehyde in acetate buffer at pH = 5, room temperature and a reactant concentration of 1 mM (stoichiometry 1:1). The reaction was followed by LCMS, analyzing the conversion rate by integration of the peaks at 565 nm (characteristic for sulforhodamine B). The resulting second order rate constant (k) is 0.32 $M^{-1}s^{-1}$ (SI Figure 1). Saito et al. had shown a strong dependence of the Pictet Spengler reaction rate depending on the nature of the aldehyde[15] (published reaction rates for hydroxylamine *iso*-Pictet Spengler with isobutyraldeyde are 2-10 $M^{-1}s^{-1}$ .The reaction with an aldehyde containing peptide displayed the significantly lower constant of 0.015 $M^{-1}s^{-1}$, measured at 60 °C). For this reason, we repeated the experiment conjugating **21** to the formyl glycine containing peptide **1.** Indeed, in this experiment we obtained a lower rate constant: k = 0.05 $M^{-1}s^{-1}$ (SI Figure 2). Aldehyde reactivity towards nucleophilic attack is mainly controlled by steric and electronic factors. Since both aldehydes tested here are aliphatic, one might consider the slower reaction rate a consequence of the steric hindrance caused by the peptide. Compared to classical Pictet Spengler under physiological conditions (k = 6.25 x 10^{-4} $M^{-1}s^{-1}$)[16] our PAPS rate constant still represents an improvement of 2 orders of magnitude and also an improvement compared to the hydroxylamine *iso*-Pictet Spengler in a biomolecule context. Although an exact comparison between our PAPS reaction rates (measured at pH = 5 and room temperature) and the other published Pictet Spengler reactions rates (measured at pH between 4 and 6 and temperatures between 25 and 60 °C) is impossible to describe due to the different conditions used, we considered our rate constants fast enough to try the use of PAPS conjugation in a more complex system, such as the conjugation to an antibody.

The aldehyde tag derived from human arylsulfatase A (LCTPSRAALLTGR)[7b, 17] was genetically fused to the C-terminus of a monoclonal antibody (anti-thyroid stimulating hormone (TSH)). The antibody was co-translationally modified by the co-expression of the human sulfatase-modifying factor 1 (SUMF1, a formylglycine-generating enzyme), which converts the cysteine comprised in the aldehyde tag sequence into formylglycine (LCTPSRAALLTGR -> L*formylglycine*TPSRAALLTGR)[7a]. After purification, this formylglycine containing antibody (**AB-CHO**) was conjugated at 37 °C for 24 h with a 5-fold excess of biotinylated pyrrolyl alanine **19** (in 100mM Na-citrate buffer with 150 mM NaCl at pH 5; Figure 4a) affording biotinylated antibody **AB(PAPS)Bi**. As control experiment, a similar antibody (**AB**), comprising the sequence LATPSRAALLTGR, i.e. not containing the FGE-modifiable cysteine, was incubated under the same conditions with a 100-fold excess of **19** (Figure 4b). The rate of IgG biotinylation was assessed by complex formation with fluorescein-labeled streptavidin (SA-FLUO; absorbs at 280 and 494 nm). Successful conjugation of the formylglycine containing antibody after 24 h with the *N*-pyrrolyl alanine biotin label was observed by the presence of additional peaks at 3.981, 4.373 and 5.057 min retention time on a GFC300 analytical -column corresponding to IgG-biotin/SA-FLUO complexes absorbing light also at 494 nm in addition to 280 nm (Figure 4b and SI Fig. 3). In constrast, the non-formylglycine containing control IgG did not form any complexes with SA-FLUO and only unconjugated IgG was observed as a single peak on the same analytical SEC column at a retention time of 5.348 min absorbing light only at 280 nm but not 494 nm (Fig. 4c and SI Fig. 4). This suggests that the *N*-pyrrolyl alanine biotin label has reacted specifically and almost quantitatively with the aldehyde containing antibody. To further prove the conjugation, we performed one more conjugation of the same antibody with another pyrrolyl alanine PEG biotin derivative. MALDI-TOF MS data are provided in the Supporting Information (SI Fig.5). The functionality of the **AB(PAPS)Bi** was tested in the TSH Elecsys® immunoassay (Roche Diagnostics) on a **cobas** e170 module (schematic representation of the assay setup in Figure 4c) in direct comparison with a commercial standard TSH antibody-biotin conjugate, biotinylated by random conjugation with NHS esters on lysines (**AB(amide)Bi)** and an formylglycin containing TSH antibody labeled at the aldehyde by oxime ligation with an aminooxy biotin label (**AB(oxime)Bi**) (Fig. 4d) Interestingly, both site-spefically labeled **AB(PAPS)Bi** and **AB(oxime)Bi** showed higher signals than the original commercial TSH assay. This might be caused by several factors, including a better orientation of the site-specific conjugates on a bead and the full integrity of all lysines in the antibody binding site, which is not guaranteed following random NHS conjugation. Furthermore, to assess the stability

10.1002/anie.201814200

WILEY-VCH

COMMUNICATION

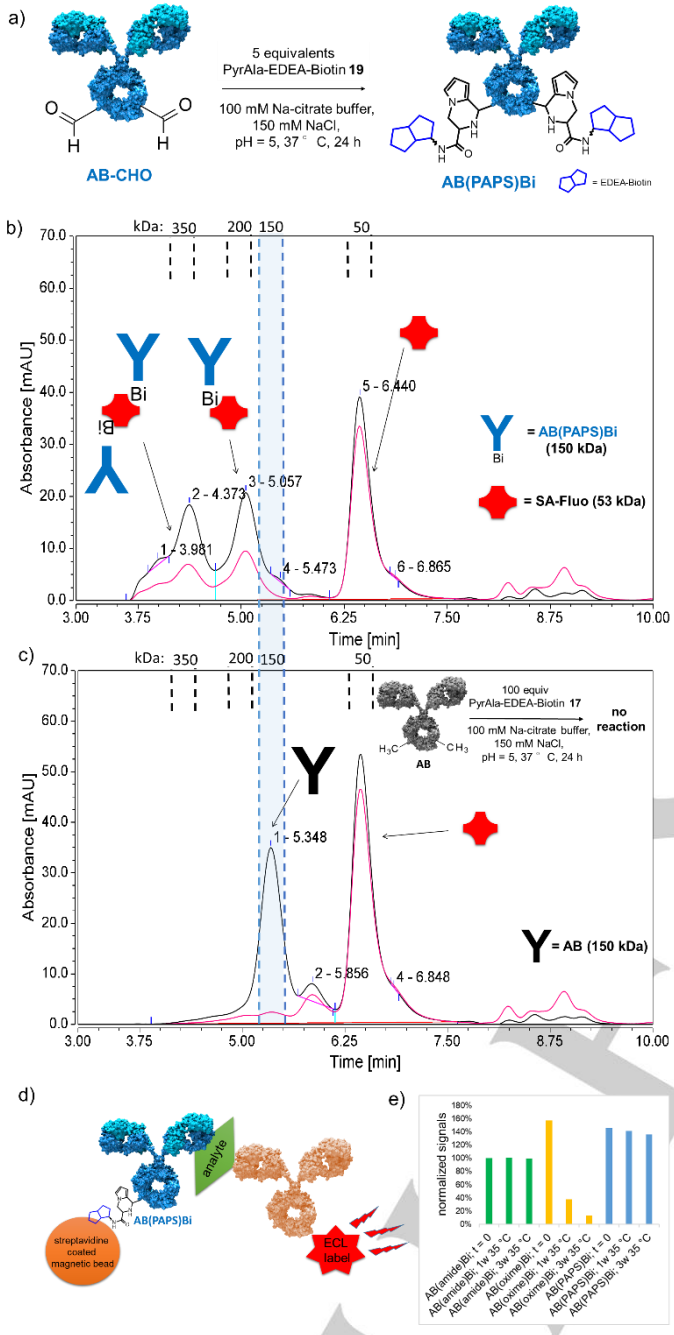


Figure 4. a) Scheme of the biotinylation of an TSH mouse IgG containing the aldehyde tag (**AB-CHO**) peptide sequence (100 mM Na-citrate buffer, 150 mM NaCl, pH = 5, 37 °C, 24 h). b) SA-Fluo analysis of the IgG containing the formylglycine, which shows the quantitative conversion to the biotinylated product (black line = absorption at 280 nm; red line = absorption at 494 nm). The double peak of the product derives from the different IgG-SA complexes, caused by the combination of streptravidin binding sites and biotinylated-IgG. c) Negative control: SA-Fluo analysis of the TSH mouse IgG not containing an aldehyde (**AB**). After incubation with a 100-fold excess of pyrrolyl alanine labeling reagent (same conditions as a) no reaction could be observed. d) Schematic representation of the **cobas** Elecsys sandwich immunoassay. e) Elecsys measurements using a commercial biotinylated antibody **AB(amide)Bi** (conjugated by NHS chemistry), an antibody biotinylated by oxime formation (**AB(oxime)Bi**) and the PAPS biotinylated antibody (**AB(PAPS)Bi**), at t = 0, after one and after three weeks incubation at 35°C. The measurements confirm the functional integrity of the PAPS labeled antibody and show good stability of the PAPS linkage.

of the *N*-pyrrolyl alanine biotin conjugates all immunoassay measurements were also done after incubation of the conjugates at 35°C for one week and for three weeks. **AB(PAPS)Bi** showed only slightly decreased signals after incubation at 35°C for one and for three weeks similar to the original assay variant with **AB(amide)Bi** conjugates suggesting that those conjugates were largely stable. In stark contrast, signal loss for more than 80% could be measured in the assay variant with the **AB(oxime)Bi** conjugates suggesting a strong decomposition (Fig. 4d). Additionally, complex formation **AB(PAPS)Bi** conjugates with SA-FLUO have been reassessed after three weeks incubation of the conjugate at 35°C. Analytical GFC300 SEC-profiles were nearly indistinguishable from treated and nontreated conjugates, indicating that there is no detectable deconjugation (Figure 4b and SI Figure 3). To further explore the versatility of *N*-pyrrolyl alanine as bioconjugation reagent we envisioned to use PAPS as ligation reaction for nucleic acid derivatives. We chose the condensation of a hexaphosphate-cytidine to an oligonucleotide tag. Hexaphosphate-oligonucleotide conjugates had been described by Fuller et al.[19] and were obtained by copper (I)-catalyzed alkyne-azide cycloaddition (CuAAC) click chemistry and used for nanopore sequencing applications. With our model reaction we wanted to examine the possibility to substitute the CuAAC reaction by PAPS conjugation, thus avoiding the use of copper catalyst, which tightly binds to the phosphate groups, and has been shown to accelerate the decomposition of the hexaphosphate moiety, if not thoroughly removed by additional EDTA washing steps. Amino undecanol hexaphosphate cytidine was synthesized according to literature procedures[19] and further functionalized with 4-formylbenzoate NHS-ester (**26**). A decathymidine oligo nucleotide with a terminal hexynyl amino linker was reacted with **16** directly on the solid synthesis support (CPG – controlled pore glass) and subsequently deprotected with diethylamine and conc. ammonia, leading after dialysis to the unprotected pyrrolyl alanine-decathymidine (Figure 5a). Aldehyde **28** and the pyrrolyl alanine modified oligonucleotide **25** were dissolved at a concentration of 0.5 mM in acetate buffer at pH = 5.4 and the reaction afforded smoothly the desired Pictet Spengler ligation product **29** (Figure 5b). Although several other methodologies for post-synthetic nucleic acid modification[20], e.g. the significantly faster tetrazine-cyclooctene ligation[21] or copper free strain promoted azido alkyne cycloadditions[22] have been successfully applied, PAPS represents a novel useful instrument in this “toolbox”, especially appealing for the good combination of the easy accessibility of the reactive moieties, reasonable reaction rate and the small and hydrophilic conjugation moiety formed upon reaction.

10.1002/anie.201814200

WILEY-VCH

COMMUNICATION

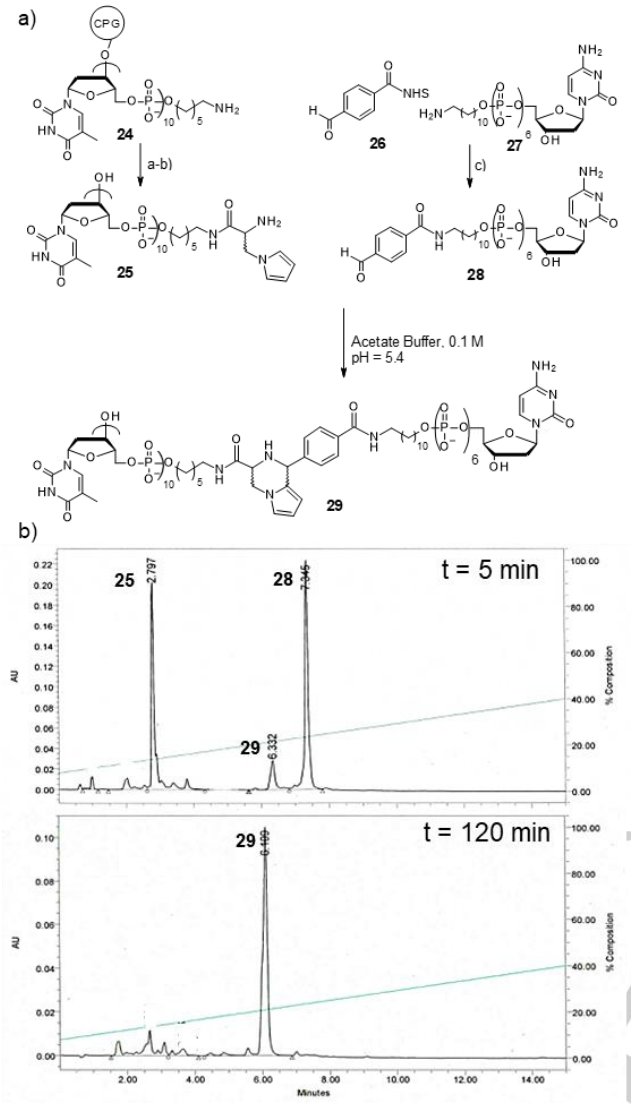


Figure 5. a) PAPS ligation of decathymidine-PyrAla with an aldehyde functionalized hexaphosphate-2'deoxycytidine. **Reagents and conditions**: *a)* **16**, DIPEA, DMF, 1h, r.t.; *b)* Et_2NH, MeCN, 10 min, NH_3 conc. 2 h, r.t.; *c)* Na-borate buffer, 0.1 M, pH = 8.5, 3 h, r.t., 46 %; PAPS conditions: pH = 5.4, Acetate buffer 0.1 M, reagent concentration = 2 mM.. b) HPLC analysis of the crude reaction mixture at t = 5 min and t = 120 min (after vivaspin centrifugation).

In summary we identified *N*-pyrrolyl alanine as an efficient reagent for fast and biocompatible Pictet Spengler conjugations. The straightforward synthesis of *N*-pyrrolyl alanine is a great advantage over other bio-Pictet Spengler reagents. Furthermore, the small size of pyrrolyl alanine and the small resulting PAPS linkage guarantees a minimal perturbation of the native biological system. PAPS was used for the site selective biotinylation of a TSH antibody obtaining quantitatively labeled antibodies with high stability, which in a **cobas** Elecsys® immunoassay performed better than the commercial product. Also, the condensation of a hexaphosphate nucleic acid derivative with an oligonucleotide yielded smoothly the desired conjugate, suggesting PAPS as a valid, catalyst free, ligation reaction for a wide range of biocompatible applications.

Acknowledgements

We wish to acknowledge the help provided by Bernhard Roettig and Annika Frauenstein by establishing the aldehyde tag methodology in our laboratories.

Keywords: protein modification • Pictet Spengler • oligonucleotide modification • aldehydes • antibody conjugation

[1] a) Craig S. McKay, M. G. Finn, *Chemistry & Biology*, *21*, 1075-1101; b) M. King, A. Wagner, *Bioconjugate Chemistry* **2014**, *25*, 825-839.
[2] a) C. R. Behrens, B. Liu, *mAbs* **2014**, *6*, 46-53; b) P. Agarwal, C. R. Bertozzi, *Bioconjugate Chemistry* **2015**, *26*, 176-192.
[3] R. J. Spears, M. A. Fascione, *Organic & Biomolecular Chemistry* **2016**, *14*, 7622-7638.
[4] K. F. Geoghegan, J. G. Stroh, *Bioconjugate Chemistry* **1992**, *3*, 138-146.
[5] a) J. M. Gilmore, R. A. Scheck, A. P. Esser-Kahn, N. S. Joshi, M. B. Francis, *Angewandte Chemie International Edition* **2006**, *45*, 5307-5311; b) R. A. Scheck, M. T. Dedeo, A. T. Iavarone, M. B. Francis, *Journal of the American Chemical Society* **2008**, *130*, 11762-11770; c) L. S. Witus, T. Moore, B. W. Thuronyi, A. P. Esser-Kahn, R. A. Scheck, A. T. Iavarone, M. B. Francis, *Journal of the American Chemical Society* **2010**, *132*, 16812-16817.
[6] a) M. Rashidian, J. M. Song, R. E. Pricer, M. D. Distefano, *Journal of the American Chemical Society* **2012**, *134*, 8455-8467; b) I. Chen, M. Howarth, W. Lin, A. Y. Ting, *Nature Methods* **2005**, *2*, 99.
[7] a) I. S. Carrico, B. L. Carlson, C. R. Bertozzi, *Nature Chemical Biology* **2007**, *3*, 321; b) P. Wu, W. Shui, B. L. Carlson, N. Hu, D. Rabuka, J. Lee, C. R. Bertozzi, *Proceedings of the National Academy of Sciences* **2009**, *106*, 3000-3005; c) J. E. Hudak, H. H. Yu, C. R. Bertozzi, *Journal of the American Chemical Society* **2011**, *133*, 16127-16135; d) J. E. Hudak, R. M. Barfield, G. W. de Hart, P. Grob, E. Nogales, C. R. Bertozzi, D. Rabuka, *Angewandte Chemie International Edition* **2012**, *51*, 4161-4165; e) D. Rabuka, J. S. Rush, G. W. deHart, P. Wu, C. R. Bertozzi, *Nature Protocols* **2012**, *7*, 1052.
[8] a) X. Ning, R. P. Temming, J. Dommerholt, J. Guo, D. B. Ania, M. F. Debets, M. A. Wolfert, G.-J. Boons, F. L. van Delft, *Angewandte Chemie International Edition* **2010**, *49*, 3065-3068; b) J. Alam, T. H. Keller, T.-P. Loh, *Journal of the American Chemical Society* **2010**, *132*, 9546-9548; c) J. Alam, T. H. Keller, T.-P. Loh, *Chemical Communications* **2011**, *47*, 9066-9068; d) M.-J. Han, D.-C. Xiong, X.-S. Ye, *Chemical Communications* **2012**, *48*, 11079-11081; e) P. I. Kitov, D. F. Vinals, S. Ng, K. F. Tjhung, R. Derda, *Journal of the American Chemical Society* **2014**, *136*, 8149-8152; f) P. Wang, S. Zhang, Q. Meng, Y. Liu, L. Shang, Z. Yin, *Organic Letters* **2015**, *17*, 1361-1364; g) J. Yu, D. Shen, H. Zhang, Z. Yin, *Bioconjugate Chemistry* **2018**, *29*, 1016-1020; h) T. S. Howard, R. D. Cohen, O. Nwajiobi, Z. P. Muneeswaran, Y. E. Sim, N. N. Lahankar, J. T. H. Yeh, M. Raj, *Organic Letters* **2018**; i) R. J. Spears, R. L. Brabham, D. Budhadev, T. Keenan, S. McKenna, J. Walton, J. A. Brannigan, A. M. Brzozowski, A. J. Wilkinson, M. Plevin, M. A. Fascione, *Chemical Science* **2018**, *9*, 5585-5593.
[9] D. K. Kölmel, E. T. Kool, *Chemical Reviews* **2017**, *117*, 10358-10376.
[10] T. Sasaki, K. Kodama, H. Suzuki, S. Fukuzawa, K. Tachibana, *Bioorganic & Medicinal Chemistry Letters* **2008**, *18*, 4550-4553.
[11] a) P. Agarwal, J. van der Weijden, E. M. Sletten, D. Rabuka, C. R. Bertozzi, *Proceedings of the National Academy of Sciences* **2013**, *110*, 46-51; b) P. Agarwal, R. Kudirka, A. E. Albers, R. M. Barfield, G. W. de Hart, P. M. Drake, L. C. Jones, D. Rabuka, *Bioconjugate Chemistry* **2013**, *24*, 846-851.
[12] P. Thompson, B. Bezabeh, R. Fleming, M. Pruitt, S. Mao, P. Strout, C. Chen, S. Cho, H. Zhong, H. Wu, C. Gao, N. Dimasi, *Bioconjugate Chemistry* **2015**, *26*, 2085-2096.
[13] T. Ohya, M. Niitsu, *Biological and Pharmaceutical Bulletin* **2003**, *26*, 1215-1218.
[14] J. Rush, C. R. Bertozzi, *Organic Letters* **2006**, *8*, 131-134.
[15] F. Saito, H. Noda, J. W. Bode, *ACS Chemical Biology* **2015**, *10*, 1026-1033.
[16] J. J. Maresh, L.-A. Giddings, A. Friedrich, E. A. Loris, S. Panjikar, B. L. Trout, J. Stöckigt, B. Peters, S. E. O'Connor, *Journal of the American Chemical Society* **2008**, *130*, 710-723.
[17] S. R. Hanson, M. D. Best, C.-H. Wong, *Angewandte Chemie International Edition* **2004**, *43*, 5736-5763.
[18] W. Steffen, F. C. Ko, J. Patel, V. Lyamichev, T. J. Albert, J. Benz, M. G. Rudolph, F. Bergmann, T. Streidl, P. Kratzsch, M. Boenitz-Dulat, T.

10.1002/anie.201814200

Oelschlaegel, M. Schraeml, *Journal of Biological Chemistry* **2017**, *292*, 15622-15635.
[19] C. W. Fuller, S. Kumar, M. Porel, M. Chien, A. Bibillo, P. B. Stranges, M. Dorwart, C. Tao, Z. Li, W. Guo, S. Shi, D. Korenblum, A. Trans, A. Aguirre, E. Liu, E. T. Harada, J. Pollard, A. Bhat, C. Cech, A. Yang, C. Arnold, M. Palla, J. Hovis, R. Chen, I. Morozova, S. Kalachikov, J. J. Russo, J. J. Kasianowicz, R. Davis, S. Roever, G. M. Church, J. Ju, *Proceedings of the National Academy of Sciences* **2016**, *113*, 5233-5238.
[20] For examples see: a) Merkel, M. , Peewasan, K. , Arndt, S. , Ploschik, D. and Wagenknecht, H., *ChemBioChem* **2015**, 16, 1541-1553; b) Rieder, U. and Luedtke, N. W., *Angew. Chem. Int. Ed*. **2014**, 53: 9168-9172. c) Juliane Schoch, Manfred Wiessler, and Andres Jäschke, *Journal of the American Chemical Society* **2010** *132* (26), 8846-8847
[21] Ke Wang, Danzhu Wang, Kaili Ji,Weixuan Chen, Yueqin Zheng, Chaofeng Dai, Binghe Wan, *Org. Biomol. Chem.* **2015**,13, 909-915
[22] a) C.-X. Song,K.E.Szulwach, Y. Fu, Q. Dai, C. Yi,X.L i, Y. Li, C.-H. Chen,W . Zhang, X. Jian, J. Wang, L. Zhang, T. J. Looney,B.Zhang, L. A. Godley, L. M. Hicks, B. T. Lahn, P. Jin, C. He, Nat. Biotechnol. 2011, 29,68–72; b) Isaac S. Marks, Jun Sung Kang, Brady T. Jones, Kevin J. Landmark, Andrew J. Cleland, and T. Andrew Taton, *Bioconjugate Chemistry* **2011** *22* (7), 1259-1263; Montserrat Shelbourne, Xiong Chen, Tom Brown, Afaf H. El-Sagheer, *Chem. Commun.* **2011**, 47, 6257-6259.

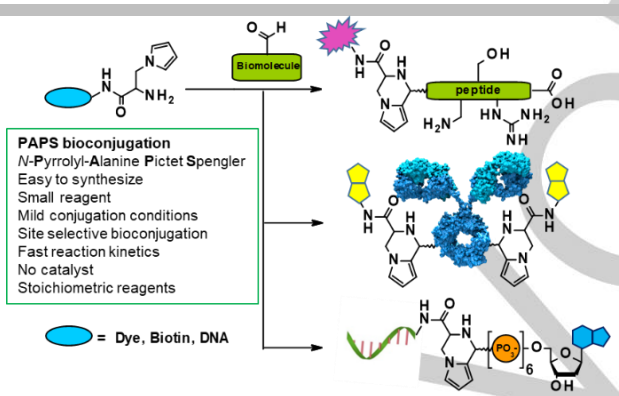
10.1002/anie.201814200

WILEY-VCH

Table of Contents

COMMUNICATION

Small but mighty: the atom efficient and easy to synthesize reagent *N*-pyrrolyl alanine is shown to undergo fast and site selective Pictet Spengler bioconjugation. This catalyst free conjugation strategy was successfully applied for the biocompatible, site specific and stable labeling of aldehydes contained in peptides, antibodies and nucleic acids.



Sebastian Pomplun, Mohamed YH Mohamed, Tobias Oelschlaegel, Christian Wellner, Frank Bergmann*

Page No. – Page No.

Efficient Pictet Spengler Bioconjugation with *N*-Substituted Pyrrolyl Alanine Derivatives