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# Biotin Labelling of Amines by Polymer-Assisted Solution-Phase Synthesis

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Dedicated to Prof. Dr. J. Thiem on the occasion of his 60th birthday

**Abstract**—An efficient and simple polymer-assisted approach has been developed to biotinylate multifunctional compounds bearing an amino functionality. Biotin was immobilized on aminomethylated polystyrene via the Kenner safety catch linker, activated and subsequently transferred to the amino function of the target compounds chemoselectively, thus avoiding protecting group operations. This approach holds potential for the introduction of spacer-modified biotin derivatives. 

## Introduction

Biotinylated compounds represent useful tools for the study of glycoprotein interactions with receptors and proteins based on the observation that low- or high-molecular-weight molecules coupled to biotin can still be recognized by avidin or streptavidin. Only the bicyclic ring system of the biotin molecule is essential for the recognition by these biotin-binding proteins. Modifications in the carboxyl group of the valeric acid side chain can therefore lead to the design of biotinyl derivatives with retained molecular recognition capability. Thus, biotin labelling is applied in a wide variety of bioanalytical applications, such as affinity chromatography, affinity cytochemistry, and immunoassays.[1] Recently, the application of the avidin–biotin or streptavidin–biotin system has been extended to novel applications, for example for drug development using biosensors.[2]

Several methods are currently available for the biotinylation of amines, imidazoles, phenols, sulfhydryls, aldehydes, and functional groups on biomolecules. These methods include in many cases quantitative reactions, but the need for protecting group operations as well as the separation of the product from excess reagent and by-products (by e.g., HPLC, dialysis, or affinity chromatography) are often time-consuming.

Herein, we describe a new simple and efficient method to covalently label multifunctional amines with biotin avoiding such protecting group operations.

## Chemistry

A polymer-bound, chemoselective biotinylation reagent was synthesized applying the safety catch linker introduced by Kenner et al.[3] Originally developed for peptide synthesis, this sulfonamide-based linker had been reinvented and adapted for solid-phase organic synthesis by Backes et al., and recently applied to polymer-assisted solution-phase (PASP) synthesis by our group.[4–6] Biotin was coupled to the sulfamoyl group of the linker molecule attached to aminomethylated polystyrene resin via in situ anhydride formation. The biotin-loaded sulfonamide linker was alkylated with bromoacetonitrile yielding polymer-bound biotin **2** as an activated species that displayed significant chemoselectivity for the acylation of amino groups. In our experiments **2** proved to be ideally suited for the polymer-supported biotin labelling of the nucleoside derived amines **1a–c** because of its absence of reactivity towards primary and secondary hydroxyl groups. Moreover secondary *N*-acylsulfonamides have just been disclosed as valuable tools for the chemical discrimination between amino groups with different properties in terms of steric hindrance.[7] As a result, no protecting groups were necessary during the biotinylation of **1a–d**. $^1$H NMR and MPLC analysis revealed high purity of the products

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obtained, and demonstrated the absence of impurities other than starting material (**1a–d**) or minor quantities of biotin hydrolyzed from the resin (Fig. 1, Table 1).

## Results and Discussion

The activated biotin was transferred to the primary amino group of **1a–d** by agitation in an appropriate solvent chemoselectively, yielding target compounds **3a–d** with high to quantitative conversion rates. PASP synthesis generally leads to simple product isolation because the excess of polymer-bound reagent that is necessary to drive the reaction to completion can be removed by filtration. This allows for the convenient biotinylation of structurally diverse amines, even for non-trained personnel staff or scientists from other fields with little education in chemistry.

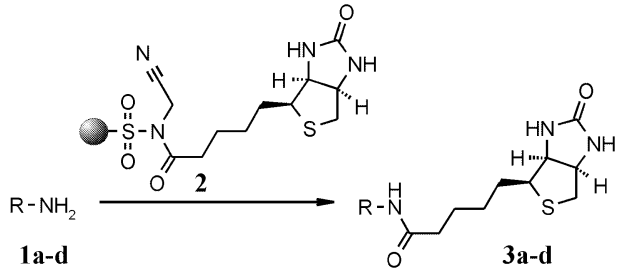



**Figure 1.** PASP synthesis of biotinylated compounds **3a–d**.

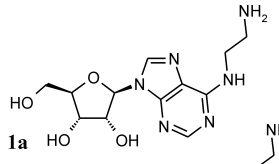
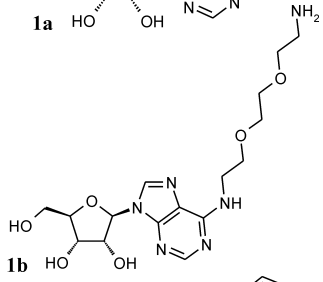
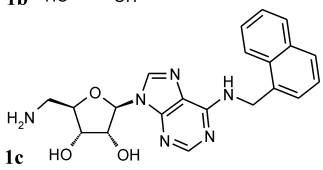
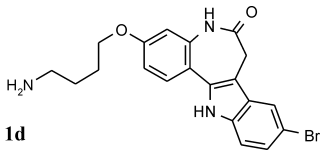
The fact that the yields observed were lower than the conversion rates has to be assigned to handling losses during filtration steps as well as absorption phenomena. This technical problem is partly attributed to the small scale (10 μmol) of the syntheses described, especially the quantitative transfer of purified compounds to balanced containers for weighing purposes in dry state is difficult. Nevertheless, the biotinylation of tiny amounts of valuable scaffolds is dependably possible using this approach.

Compound **3d** will be attached to a commercially available streptavidin-charged gold chip via streptavidin–biotin interaction. This will enable an envisioned investigation by surface plasmon resonance analysis. Employing a Biacore™ system, **3d** will be used to screen for hitherto not identified tissue components like proteins or cellular factors to which this novel paullone derivative may bind. In this way, we seek to gain further insights in the mode of action of this highly promising class of compounds.[8]

The protocol described is not only limited to biotinylation itself, but could also be applied to other labelling techniques. Photoaffinity tags and fluorescence dyes, for example are gaining increasing interest for labelling biomolecules such as peptides and oligonucleotides for investigation of biological materials.[9,10]

In intact cells, recognition of photolabelled glucose transporters by avidin/streptavidin only occurs if a spacer with a minimum of 60–70 spacer atoms between

**Table 1.** Conversion, yields and purity for biotinylated compounds **3a–d**

| Product | From R-$NH_2$ (**1a–d**) | Conversion[a] (%) | Yield[b] (%) | Purity[c] (%) |
|---|---|---|---|---|
| **3a** |  **1a** | 98 | 95 | 98 |
| **3b** |  **1b** | 100 | 97 | 99 |
| **3c** |  **1c** | 96 | 82 | 87 |
| **3d** |  **1d** | 87 | 80 | 87 |

[a]Conversion as ratio of product to sum of unchanged starting material and product calculated from MPLC-purification profile.
[b]Isolated purified material.
[c]Semipreparative MPLC, 100% method, detection at 254 nm.

the biotin and the photoreactive moiety is introduced.[10] Thus, depending on the localization of the membrane protein to be analyzed, biotinylated photoaffinity compounds require a custom-tailored length of the spacer. PASP synthesis offers a simple possibility to introduce different spacers of various lengths via on-bead construction of amide chains using established amide bond forming procedures in a short period of time. Currently, our group is addressing this issue, and the results will be reported in due course.

In conclusion, PASP synthesis was efficiently employed for the biotinylation of four amines leading to the desired labelled products in high conversion rates, good yields and purity. The procedure proved to be simple, reliable and efficient.

## Experimental

Identity of all compounds was assigned by NMR spectroscopy. Sample purity was calculated from chromatographic purification profiles. Yields are reported as isolated material. $^1$H NMR spectra were recorded on a Bruker AMX 400 spectrometer at 400 MHz in DMSO-$d_6$, using tetramethylsilane as internal standard. MPLC simultaneous purity analyses/purifications were performed using a Büchi 681 pump (flow rate 10 mL, MeOH/$H_2O$ 30:70), and UV-detector (254 nm) with Merck 310-25 Lobar-LiChroprep$^{TM}$-RP-18 columns. MS data of pure compounds was obtained on a Finnigan MAT 311A (FAB) or XL 95 (ESI) instrument, respectively.

Biotinylation was carried out according to procedures reported for other carboxylic acids recently with the following deviations: 20 mL of DMF were added to 2.0 g of dry 4-sulfamylbenzoylaminomethyl polystyrene with an initial loading level of 1.2 mmol/g.[5,6] The resin was allowed to swell at room temperature for 2 h. Separately, 10 mmol of biotin was dissolved in 30 mL DMF (50 °C) and preactivated via in situ anhydride formation by adding 780 μL (5 mmol) *N,N*-diisopropylcarbodiimide. After addition of 580 μL *N*-ethyl-*N,N*-diisopropylamine (DIPEA) (3.4 mmol) and 15 mg of 4-(dimethyl)aminopyridine (0.12 mmol) as catalyst to the swollen resin, the coupling mixture was added. The resulting reaction mixture was shaken at room temperature for 24 h. The resin beads were filtered off and washed exhaustively with DMF, dichloromethane and methanol, three times 5 mL, each.

The sulfonamide linker (400 mg) was then activated for cleavage by alkylation with 640 μL of bromoacetonitrile (9 mmol) and 340 μL of DIPEA (2 mmol) in 4 mL 1-methylpyrrolidone (NMP) overnight. The resin beads were washed with dimethylsulfoxide and THF, five times 5 mL and three times 5 mL, respectively.

The polymer-bound, activated biotin was transferred to the amino group of 10 μmol of **1a**–**d**, respectively, by adding a solution of **1a**–**d** in 0.5 mL NMP to a slurry of 400 mg resin **2** in 3 mL THF (**1a**,**b**,**d**) or NMP (**1c**) and shaking at 55 °C for 10 h.

**$N^6$-(2-Biotinylamido-ethyl)adenosine (3a).** $^1$H NMR δ (ppm) = 8.36 (s, 1H, 8H), 8.22 (s, 1H, 2H), 7.94 (t, 1H, NH, amide, $J$ = 5.60 Hz), 7.85 (bs, 1H, $N^6$H), 6.41 (s, 1H, NH, biotin), 6.35 (s, 1H, NH, biotin), 5.89 (d, 1H, 1′H, $J$ = 6.10 Hz), 5.45 (d, 1H, 3′OH, $J$ = 6.10 Hz), 5.42 (dd, 1H, 5′OH, $J$ = 4.58, 2.55 Hz), 5.20 (d, 1H, 2′OH), 4.61 (dd, 1H, 2′H), 4.31–4.28 (m, 1H, 3′H), 4.16–4.09 (m, 2H, biotin), 3.98–3.95 (m, 1H, 4′H), 3.70–3.65 (m, 1H, 5′H), 3.58–3.52 (m, 3H, 5′H, ethylene), 3.08–3.03 (m, 1H, biotin), 2.82 (dd, 1H, biotin, $J$ = 5.09, 7.63 Hz), 2.58 (d, 1H, biotin, $J$ = 12.21 Hz), 2.05 (t, 2H, $J$ = 7.12, 7.63 Hz), 1.64–1.20 (m, 6H, biotin). HRFAB-MS $[M+H]^+$ calcd 537.2245, found 537.2249.

**$N^6$-[8-Biotinylamido-(3,6-dioxooctanyl)]adenosine (3b).** $^1$H NMR δ (ppm) = 8.36 (s, 1H, 8H), 8.22 (s, 1H, 2H), 7.84 (t, 1H, NH, amide, $J$ = 5.60, 5.09 Hz), 7.78 (bs, 1H, $N^6$H), 6.41(s, 1H, NH, biotin), 6.35 (s, 1H, NH, biotin), 5.89 (d, 1H, 1′H, $J$ = 6.11 Hz), 5.46 (d, 1H, 3′OH, $J$ = 6.10 Hz), 5.41 (dd, 1H, 5′OH, $J$ = 4.57, 2.55 Hz), 5.20 (d, 1H, 2′OH, $J$ = 5.08 Hz), 4.61 (q, 1H, 2′H, $J$ = 6.10, 5.09 Hz), 4.32–4.26 (m, 1H, 3′H), 4.16–4.09 (m, 2H, biotin), 3.99–3.94 (m, 1H, 4′H), 3.70–3.49 (m, 11H, 5′$H_2$, dioxooctane), 3.19–3.15 (q, 2H, dioxooctane), 3.10–3.06 (m, 1H, biotin), 2.82 (dd, 1H, biotin, $J$ = 5.09, 7.12 Hz), 2.58 (d, 1H, biotin, $J$ = 12.21 Hz), 2.05 (t, 2H, $J$ = 7.12, 7.63 Hz), 1.64–1.21 (m, 6H, biotin). HRFAB-MS $[M+H]^+$ calcd 625.2768, found 625.2784.

**5′-Deoxy-5′-biotinylamido-$N^6$-(1-naphthylmethyl)-adenosine (3c).** $^1$H NMR δ (ppm) = 8.49 (bs, 1H, $N^6$H), 8.37 (bs, 1H, 8H), 8.25 (s, 1H, 2H), 8.23 (m, 1H, aromat), 8.14 (t, 1H, $J$ = 6.11 Hz, NH, amide), 7.96–7.94 (m, 1H, aromat), 7.83–7.81 (m, 1H, $J$ = 8.63 Hz, aromat), 7.59–7.52 (m, 2H, aromat), 7.46–7.41 (m, 2H, aromat), 6.41 (s, 1H, NH, biotin), 6.33 (s, 1H, NH, biotin), 5.87 (d, 1H, 1′H, $J$ = 6.36 Hz), 5.45 (d, 1H, 3′OH, $J$ = 6.11 Hz), 5.24 (d, 1H, 2′OH, $J$ = 4.84 Hz), 5.19 (bs, 2H, $CH_2$, naphthylmethyl), 4.69–4.67 (m, 1H, 2′H), 4.30–4.26 (m, 1H, biotin), 4.12–4.09 (m, 1H, biotin), 4.07–4.03 (m, 1H, 3′H), 3.98–3.94 (m, 1H, 4′H), 3.11–3.03 (m, 2H, biotin), 2.33 (t, 2H, $J$ = 7.12 Hz, biotin), 1.66–1.21 (m, 6H, biotin). HRESI-MS $[M+Na]^+$ calcd 655.2427, found 655.2428.

**3[4-(Biotinylamido)-butoxy]-9-bromo-7,12-dihydro-indolo[3,2-*d*][1]benzazepin-6(5*H*)-one (3d).** $^1$H NMR δ (ppm) = 11.69 (s, 1H, NH, paullone), 10.02 (s, 1H, NH, paullone), 7.85 (d, 1H, $J$ = 1.52 Hz, paullone), 7.82 (t, 1H, $J$ = 5.60 Hz, NH, amide), 7.64 (d, 1H, $J$ = 8.64 Hz, paullone), 7.36 (d, 1H, $J$ = 8.12, paullone), 7.23 (dd, 1H, $J$ = 8.66, 2.02 Hz, paullone), 6.91 (dd, 1H, $J$ = 8.90, 2.30 Hz, paullone), 6.80 (d, 1H, $J$ = 2.04 Hz, paullone), 6.42 (s, 1H, NH, biotin), 6.36 (s, 1H, NH, biotin), 4.30–4.27 (m, 1H, biotin), 4.10–4.03 (m, 1H, biotin), 4.02 (t, 2H, $J$ = 6.34 Hz, paullone), 3.49 (s, 2H, paullone), 3.12–3.06 (m, 3H, 1H biotin, 2H paullone, overlapping), 2.80 (dd, 1H, $J$ = 12.72, 5.08 Hz, biotin), 2.56 (d, 1H, $J$ = 12.72 Hz, biotin), 2.05 (t, 2H, $J$ = 7.12 Hz, biotin), 1.77–1.70 (m, 2H, paullone), 1.64–1.41 (m, 6H, 4H biotin, 2H paullone, overlapping), 1.35–1.28 (m, 2H, biotin). HRFAB-MS $[M+H]^+$ calcd 640.1593, found 640.1646.

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## References and Notes

1. Wilchek, M.; Bayer, E. *Anal. Biochem.* **1988**, *171*, 1.
2. Sakai, T.; Naidenko, O.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1999**, *42*, 1836.
3. Kenner, G.; McDermott, J.; Sheppard, R. *J. Chem. Soc., Chem. Commun.* **1971**, 636.
4. Backes, B.; Ellman, J. *J. Am. Chem. Soc.* **1994**, *116*, 11171.
5. Backes, B.; Virgilio, A.; Ellman, J. *J. Am. Chem. Soc.* **1996**, *118*, 3055.
6. Golisade, A.; Bressi, J.; Van Calenbergh, S.; Gelb, M.; Link, A. *J. Comb. Chem.* **2000**, *2*, 537.
7. Kondo, K.; Sekimoto, E.; Nakao, J.; Murakami, Y. *Tetrahedron* **2000**, *56*, 5843.
8. Schultz, C.; Link, A.; Leost, M.; Zaharevitz, D.; Gussio, R.; Sausville, E. A.; Meijer, L.; Kunick, C. *J. Med. Chem.* **1999**, 2909.
9. Wojczewski, C.; Stolze, K.; Engels, J. *Synlett* **1999**, *10*, 1667.
10. Hashimoto, M.; Yang, J.; Holman, G. *ChemBioChem.* **2001**, *2*, 52.