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Novel preloaded resins for solid-phase biotinylation of carboxylic acids

Nadezda Cankarova, Petr Funk, Jan Hlavac, Miroslav Soural *

Department of Organic Chemistry, Institute of Molecular and Translational Medicine, Faculty of Science, University of Palacky, 17. listopadu 12, 771 46 Olomouc, Czech Republic

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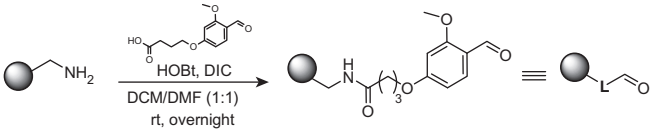
ABSTRACT

Use of solid-phase synthesis for the derivatization of carboxylic acids with biotinylated spacers consisting of ethylenoxy units is described. An aminomethylated resin provided with an acid-labile aldehyde linker is used as the polymer support and three different systems with a reactive amino group are introduced. Acylation of each system was tested with a set of model carboxylic acids and afforded crude products of excellent purity. The preloaded resins are similar to the Biotin-PEG-NovaTagTM resin but offer several advantages including simple elongation of the spacer arm. The protocols described represent a very efficient way of modifying compounds to obtain ligands for affinity chromatography studies.

Biotinylation of organic compounds is a very frequent approach for detailed studies of biochemical properties of biologically active substances. Biotinylated agents are widely used to immobilize compounds for affinity chromatography,[1,2] labeling of peptides,[3,4] proteins,[5–7] nucleic acids, antibodies,[8] etc. When the molecule to be studied is suitably derivatized with biotin, it can be simply immobilized on a chromatography column by using its highly specific and tight non-covalent interaction with avidin or streptavidin.[9] When the selected compound is modified for an affinity chromatography assay, the key task is incorporation of the spacer between the molecule to be studied and biotin.[10–12] The spacer must meet the following basic criteria: (i) it has to be long enough to avoid steric repulsion between avidin and the target biomolecule which interacts with the immobilized compound, (ii) it has to be inert to non-specific interactions with biomolecules that do not interact with the immobilized compound, (iii) it has to avoid decreasing the biotinylated system's solubility in water. Aliphatic spacers (for instance, a spacer consisting of the caproic acid skeleton) have been used for a long time. However, more recently they have been successfully replaced by ethylenoxy units—containing ligands (ethylene glycols—EGs) which fulfill the last two mentioned criteria. For applications in which a longer spacer is required (e.g., more than 12 atoms) EGs, in contrast to aliphatic chains, avoid hydrophobic interactions resulting in deformation of the spacer.

The most frequent way to attach the compound to be studied to the biotin-EG system is: (i) acylation of the biotin-EG-NH_2 system with carboxy group-containing compounds, (ii) acylation of an amino group-containing compound with the biotin-EGs-COOH system. In this Letter we describe an efficient method of biotinylation of carboxylic acids using the first mentioned method. So far, biotinylation of appropriate compounds via EG linkers has been commonly performed using solution-phase synthesis and/or commercially available biotin-EG-NH_2 systems. However, solution-phase modification of compounds is usually complicated by difficult and time-consuming isolation of products from reaction mixtures and also complicated purification of intermediates as well as of the final products. Such problems can be eliminated when the concept of solid-phase synthesis is applied. There are two alternatives to the use of solid-phase synthesis for biotinylation of organic compounds: (i) the substrate which undergoes biotinylation is pre-immobilized (or directly synthesized) on an insoluble polymer support.[13] (ii) the biotin-EG-NH_2 system is immobilized and the resulting preloaded resin is used for an acylation reaction with an appropriate carboxy group-containing compound. Quite recently, Novabiochem has introduced the first preloaded resin of this kind: Biotin-PEG-NovaTag™ resin, which has already been successfully used in practice in several cases.[14–16] In this Letter, we describe preparation and use of alternative systems which offer



Scheme 1. Attachment of an acid-labile linker to the aminomethylated resin.

* Corresponding author. Tel.: +420 585634418; fax: +420 585634465.
E-mail addresses: souralm@seznam.cz, soural@orgchem.upol.cz (M. Soural).

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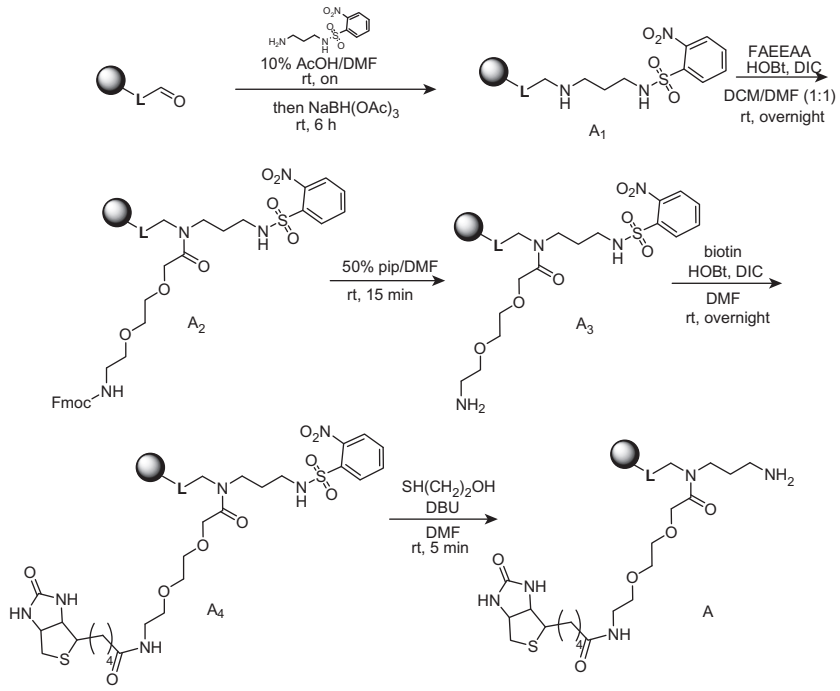
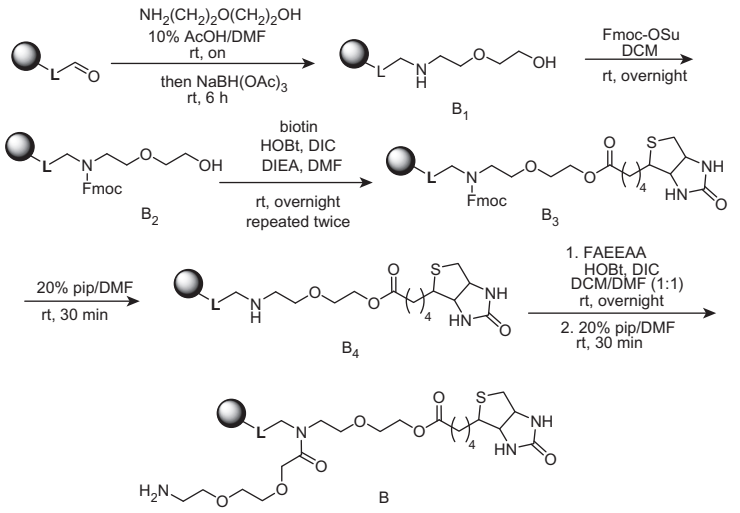


Scheme 2. Preparation of system **A**.



Scheme 3. Preparation of system **B**.

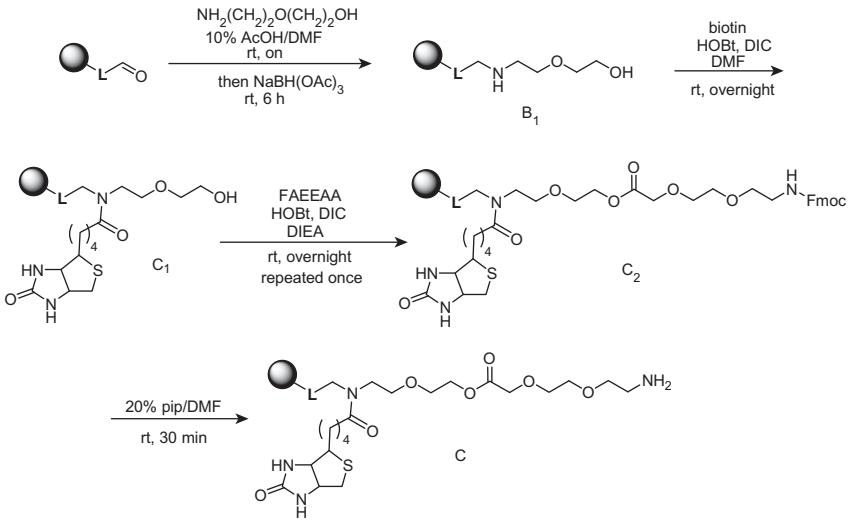
several advantages including simple elongation of the spacer arm, and can be synthesized simply with the help of developed procedures involving commercially available synthons.

Polystyrene resin was chosen as the polymer support because of the very easy isolation of reaction products it allows. This consists of only a simple filtration after each synthetic step. All the systems were prepared by using an acid-labile linker attached to the aminomethylated resin (Scheme 1).

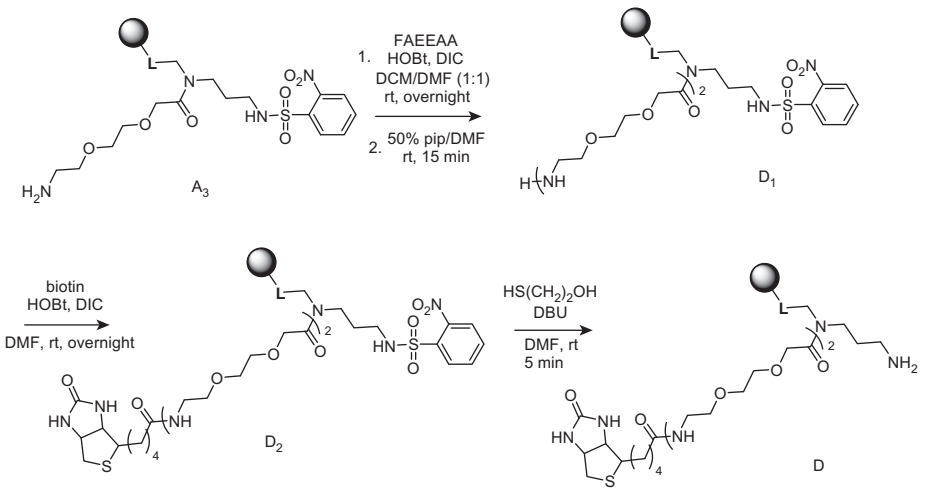
Three different systems with various lengths and structures were prepared and tested. The first step of the entire reaction sequence was the reductive amination of the benzaldehyde linker with *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide (for systems **A**) or 2-(2-aminoethoxy)ethanol (for systems **B** and **C**). The key building block for the preparation of each system was the commercially available [2-[2-(Fmoc-amino)ethoxy]ethoxy]acetic acid (FAEEAA hereafter).

(i) System **A**: Immobilized *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide **A1** was acylated with FAEEAA, the Fmoc-protective group of the intermediate **A2** was cleaved and the resulting aminoderivative **A3** was acylated with biotin. Finally, the 2-Nos-protective group of the derivative **A4** was cleaved to obtain the system **A** (Scheme 2).

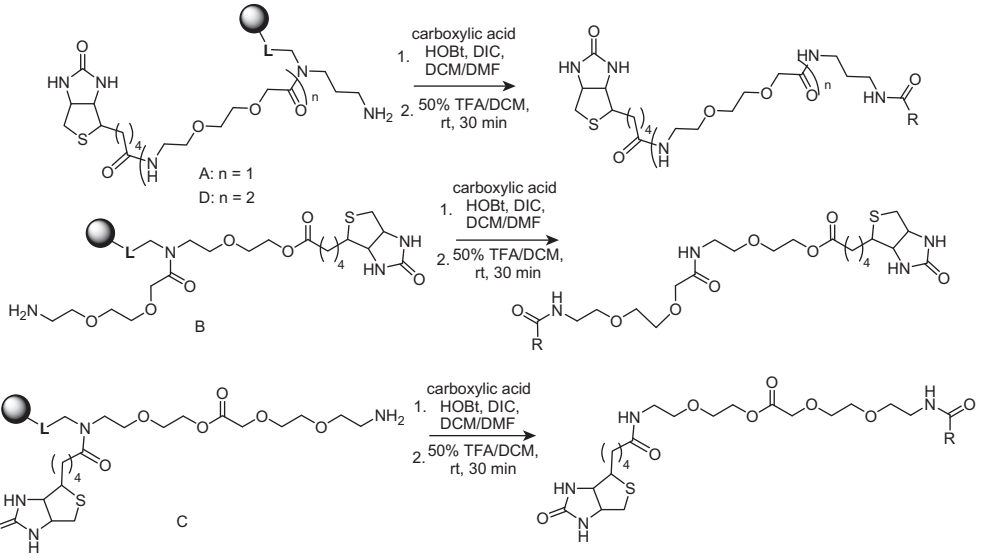
(ii) System **B**: Immobilized 2-(2-aminoethoxy)ethanol **B1** was *N*-Fmoc protected using Fmoc-OSu and the terminal hydroxy group of the intermediate **B2** was biotinylated (the reaction had to be repeated twice for quantitative esterification). Subsequently, the Fmoc-protective group was



Scheme 4. Preparation of system **C**.

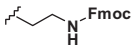
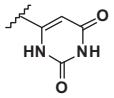
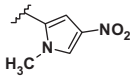
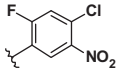


Scheme 5. Preparation of system **D**.



Scheme 6. Acylation and cleavage of final systems **A**, **B**, **C**, and **D** from the polymer support.

Table 1
Acids used for acylation of systems **A**, **B**, **C**, and **D**

R	System	Product	Purity[a] (%)
	**A**	**1**	93
	B	**2**	95
	C	**3**	97
	D	**4**	94
	**A**	**5**	88
	B	**6**	95
	C	**7**	97
	**A**	**8**	95
	B	**9**	95
	C	**10**	98
	**A**	**11**	96
	B	**12**	94
	C	**13**	97
	**A**	**14**	93
	B	**15**	92
	C	**16**	96
	D	**17**	85
	**A**	**18**	90
	B	**19**	94
	C	**20**	95

[a] Purity of crude product (calculated from HPLC-UV traces).

cleaved and the intermediate **B4** was acylated with FAEEAA. Finally, the Fmoc-protective group was cleaved to give the system **B** (Scheme 3).

(iii) System **C**: 2-(2-Aminoethoxy)ethanol **B1** was immobilized and its amino group was biotinylated to give the intermediate **C1**. Subsequently the hydroxy group was acylated with FAEEAA (the reaction had to be repeated once for a quantitative conversion). Cleavage of the Fmoc-protective group of intermediate **C2** afforded target system **C** (Scheme 4).

To increase further the diversity of the method we subsequently focused on possible elongation of the spacer arm. For such a modification we chose system **A**, which was very simply transformed to system **D** consisting of twice the number of ethyleneoxy units:

(iv) System **D : Polymer-supported** aminoderivative **A3** was acylated with FAEEAA. Following cleavage of Fmoc-protective group intermediate **D1** was biotinylated to yield derivative **D2**. Finally, the 2-Nos-protective group was cleaved to access target system **D** (Scheme 5).

Systems **A**, **B**, **C**, and **D** were obtained in very good purity (about 95%, HPLC-UV traces). Their applicability for the modification of

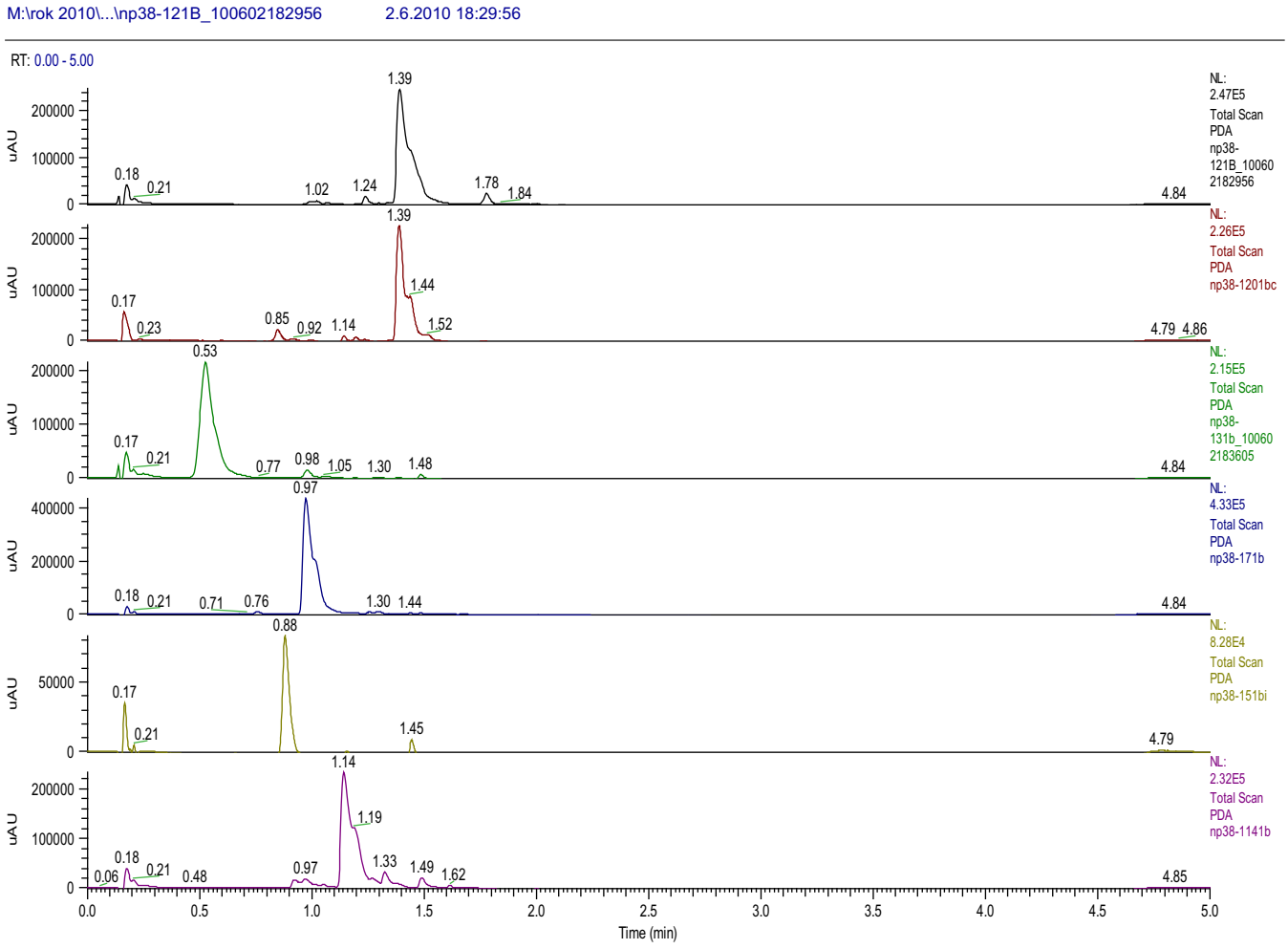


Figure 1. HPLC-UV traces of crude products **1**, **5**, **8**, **11**, **14**, and **18** obtained after acylation of system **A**.

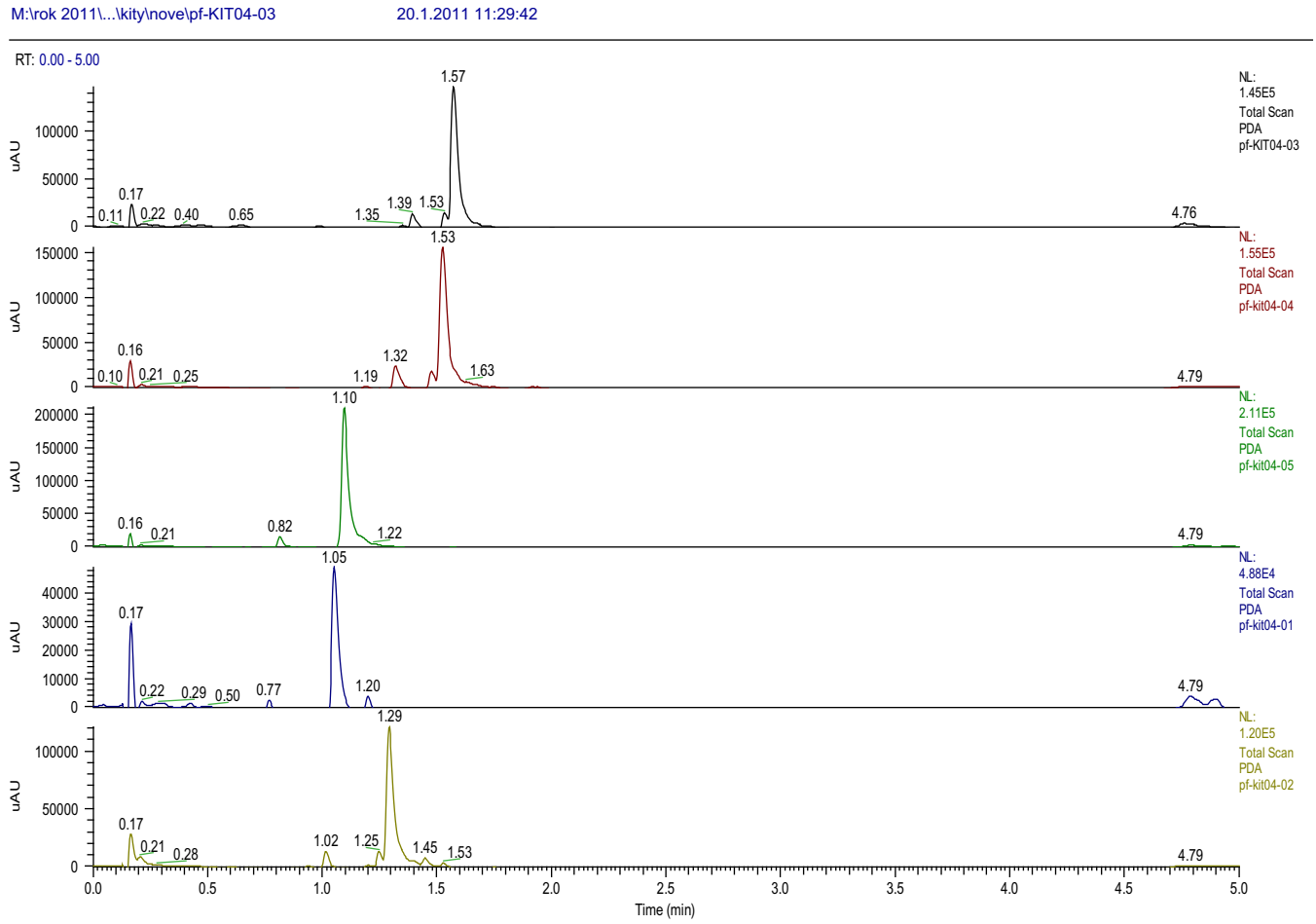


Figure 2. HPLC-UV traces of crude products **2**, **6**, **12**, **15**, and **19** obtained after acylation of system **B**.

carboxy group-containing substrates was tested using a set of model carboxylic acids. Acylation was performed with the help of in situ generated HOB*t* esters of appropriate acids (Scheme 6).

After acid-mediated cleavage from the resin, the products were obtained in excellent crude purity (Table 1). However, it must be pointed out that the method is unsuitable for modification of acid-labile compounds due to their possible decomposition during the cleavage from the polymer support. In such cases, a base-labile linker would have to be applied for the preparation of appropriate systems.

The preloaded resins introduced are an alternative solution to the use of Biotin-PEG-NovaTag™ resin. Compared to this commercially available resin, the amino group which undergoes acylation is not part of a cleavage site and this offers at least three advantages:

(i) The cleavage from the polymer support is not influenced by the character of the substrate (resonance effects can decrease the sensitivity of the amidic group towards protonation resulting in an incomplete release from the polymer support).

(ii) Evaluation of the reaction conversion can be done directly from the analytical sample of the resin with the use of HPLC-UV-(MS) traces (starting material from resins **A–D** is cleavable in contrast to Biotin-PEG-NovaTag™ resin which is uncleavable when not acylated and suitable derivatization is needed before analysis).

(iii) The distance of the amino group from the resin's surface is longer (especially in systems **B**, **C**, and **D**) which is sterically more favorable for acylation with bulkier and sterically more demanding substrates.

(iv) Finally, the most significant advantage of the method described consists of a very simple elongation of the spacer arm. With the use of the protocol described and synthons, the arm can simply be doubled (see transformation of system **A** to system **D**) but also longer systems could be synthesized if necessary.

In conclusion, we have introduced an efficient method of modifying carboxy group-containing compounds for affinity chromatography studies. The simple solid-phase synthesis methodology that was used for both the preparation and application of appropriate biotin-EG-NH_2 systems eliminates problems resulting from difficult or time-consuming isolation of products from reaction mixtures. Small quantities of appropriately modified compounds can be easily produced by the method. Furthermore, synthesis and application of systems **A**, **B**, **C**, and **D** can be performed without the need for complicated equipment (polypropylene fritted syringes were used as reaction vessels). The method was successfully tested for set of model acids and afforded the corresponding products in excellent purity without need of their further purification (see Figs. 1–4)—HPLC-UV spectra of the cleaved products) which makes the procedure theoretically applicable for routine biotinylation of a wide range of carboxylic acid derivatives.

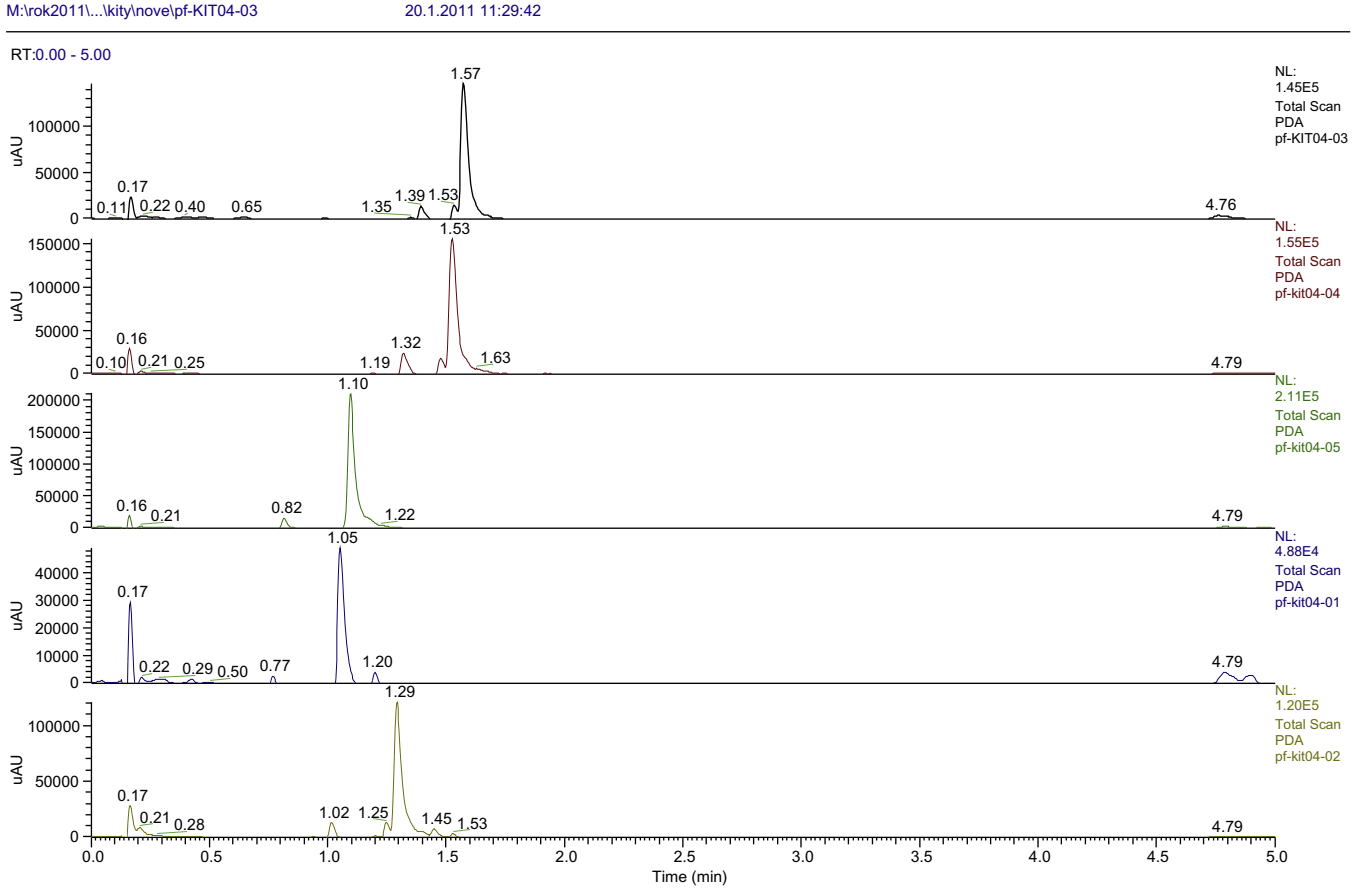


Figure 3. HPLC-UV traces of crude products **3**, **7**, **13**, **16**, and **20** obtained after acylation of system **C**.

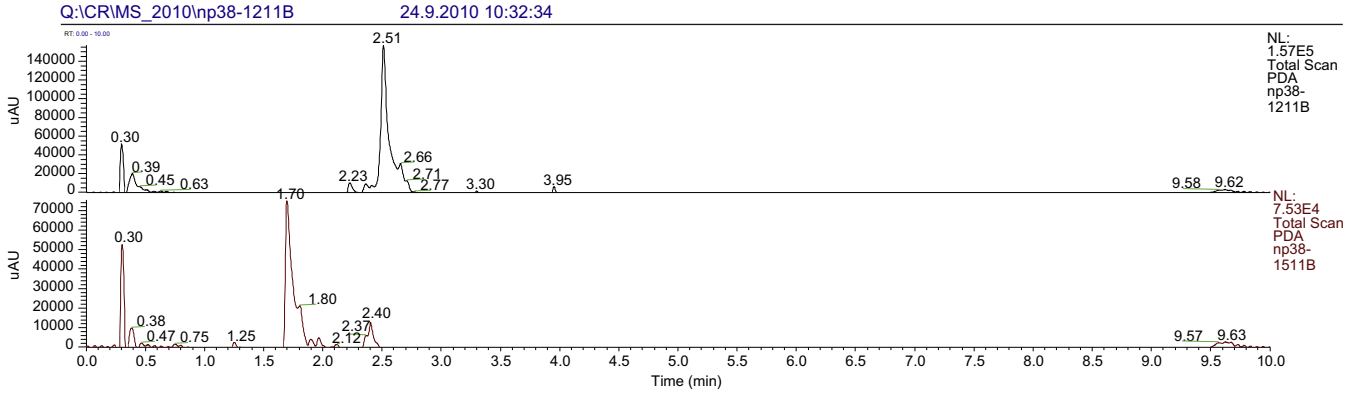


Figure 4. HPLC-UV traces of crude products **4**, and **17** obtained after acylation of system **D**.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, analytical data of syntesized compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.08.115.

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