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# A Nickel(II)-Mediated Thiocarbonylation Strategy for Carbon Isotope Labeling of Aliphatic Carboxamides

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*Dedicated to Professor Ilhyong Ryu on the occasion of his 70th birthday*

**Abstract:** A series of pharmaceutically relevant small molecules and biopharmaceuticals bearing aliphatic carboxamides have been successfully labeled with carbon-13. Key to the success of this novel carbon isotope labeling technique is the observation that $^{13}$C-labeled Ni$^{II}$-acyl complexes, formed from a $^{13}$CO insertion step with Ni$^{II}$-alkyl intermediates, rapidly react in less than one minute with 2,2′-dipyridyl disulfide to quantitatively form the corresponding 2-pyridyl thioesters. Either the use of $^{13}$C-SilaCOgen or $^{13}$C-COgen allows for the stoichiometric addition of isotopically labeled carbon monoxide. Subsequent one-pot acylation of a series of structurally diverse amines provides the desired $^{13}$C-labeled carboxamides in good yields. A single electron transfer pathway is proposed between the Ni$^{II}$-acyl complexes and the disulfide providing a reactive Ni$^{III}$-acyl sulfide intermediate, which rapidly undergoes reductive elimination to the desired thioester. By further optimization of the reaction parameters, reaction times down to only 11 min were identified, opening up the possibility of exploring this chemistry for carbon-11 isotope labeling. Finally, this isotope labeling strategy could be adapted to the synthesis of $^{13}$C-labeled liraglutide and insulin degludec, representing two antidiabetic drugs.

## Introduction

Carbon isotope chemistry plays an indispensable role in the development of new pharmaceutical entities for the treatment of inflicting human disorders. Drug development campaigns involve a number of processes, which eventually leads to a handful of compounds entering preclinical research after several years of optimization and analysis of up to thousands of molecules.[1] Subsequently, the efficacy and safety profile of these molecular entities must be tested by in vivo drug metabolism and pharmacokinetics (DMPK) providing a risk assessment of these candidates and eventually deciding on their fate as a potential commercial drug. It is precisely at this stage, where carbon isotope labelling enters the scene delivering $^{11}$C-, $^{13}$C- and $^{14}$C-labeled probes of these potential drug molecules. Whereas carbon-11 is utilized for positron emission tomography (PET) imaging studies (e.g. investigations on the propensity of a candidate to cross the blood brain barrier),[2] carbon-14 is exploited as a tracer for evaluating the drug's metabolic profile by a variety of techniques, including radioscintillation or LC-radioactivity and isotope ratio mass spectrometry techniques (IRMS).[3a–e] Later, human studies and environmental fate investigations are performed with carbon-14 labeled compounds monitoring both safety, distribution and the environmental risks of the drug.[3c,d] Carbon-13 is also useful for many of the same metabolic studies as for carbon-14,[3f] though to a lesser degree. This is explained by the easier detection of compounds labeled with carbon-14 being a radioisotope and its natural abundancy ($10^{-11}$ %), which is much lower compared to carbon-13 (1.1 %).[3e]

Strategically, it would be advantageous to develop isotope labeling technologies, which could encompass a wide range of pharmaceutically relevant compounds, including biomolecules, rather than just a handful. The question remains therefore, which of the carbon-containing functional groups would represent ideal candidates to target for the introduction of a carbon isotope? This answer can be found by reflecting on both the primary source of carbon isotope-labeled compounds, and the structural composition of some of the most important functional groups in APIs. Firstly, isotopically labeled carbon dioxide represents the starting point for most carbon-11 and carbon-14 labeled compounds.[4] Bearing in mind that reduction of labeled $CO_2$ to carbon monoxide can be achieved in a selective, fast, and efficient manner, and carbonylation chemistry is well adapted for carbonyl group installation,[5] the ideal functional

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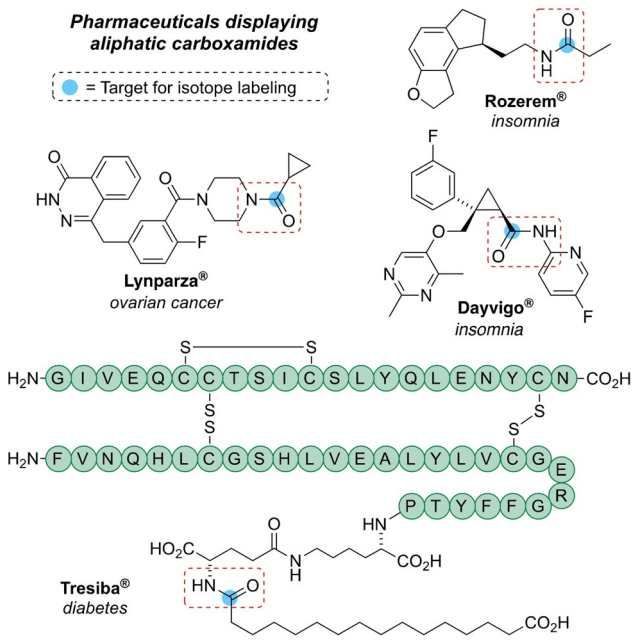
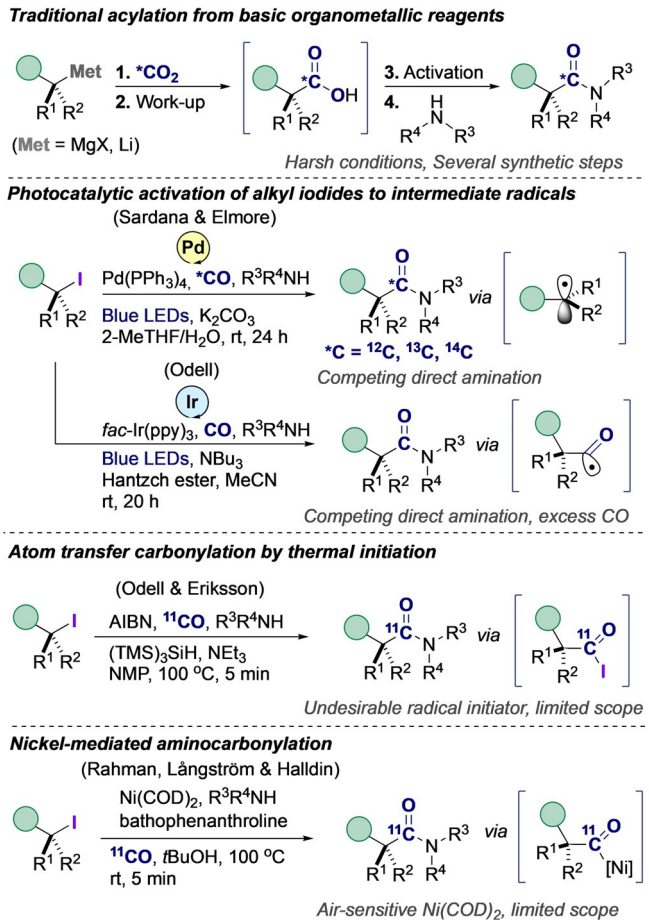



**Scheme 1.** Examples of commercial pharmaceuticals displaying aliphatic carboxamides.




**Scheme 2.** Previous synthetic strategies adaptable to carbon isotope labeling of aliphatic carboxamides.

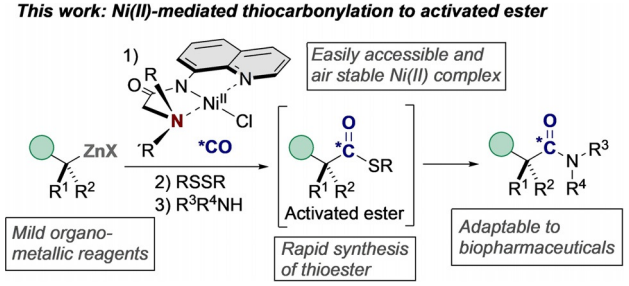
motifs for carbon isotope installment would therefore be those presenting a carbonyl entity. Secondly, analysis of the chemical structures in a recent compilation of the top 200 small molecule drugs in retail sales for 2018,[6] reveals that 79 of these contain at least one carboxamide group (Scheme 1). Furthermore, of the 34 FDA approved small molecule drugs in 2019,[7] 14 structures display a minimum of one of carboxamide, underlining the importance of this carbonyl-containing functionality even in modern-day drug development programs.

Palladium-mediated aminocarbonylations have played a principle role for the installation of carbon-11, -13 and -14 labels into carboxamide-displaying bioactive molecules because of the ability of certain Pd-complexes to promote these reactions under low CO pressures, and even under conditions with only stoichiometric or substoichiometric amounts of the isotopically labeled gas.[5] Since this chemistry exhibits excellent functional group compatibility, its adaptation for late stage isotope introduction becomes feasible with the added benefit of minimal manipulation of radioactive materials. However, the majority of these procedures are confined to the synthesis of aryl and vinyl carboxamides from the corresponding halides or activated phenols and enols.[8] Although this provides an entry to pharmaceutically related molecules carrying a benzamide or acrylamide motif, the methodology is less reliable for accessing aliphatic carboxamides because of the propensity of the intermediate alkyl palladium complex to undergo β-hydride elimination before the CO insertion step.[9] As such, a range of small and large molecule pharmaceuticals containing aliphatic amides, as depicted in Scheme 2, are excluded for late-stage isotope labeling applying this technique.

Traditionally, the carbon isotope-labeling of aliphatic carboxamides via an acylation step has been accomplished by an initial reaction of a Grignard or organolithium reagent with labeled $CO_2$, followed by generation of the corresponding activated ester (Scheme 2).[4c,10] However, limited functional group tolerance of these organometallic reagents represents a major obstacle for the adaptability of such a protocol to the isotope labeling of elaborate structures. To overcome the issues of β-hydride elimination in the Pd-catalyzed aminocarbonylation with alkyl iodides, Sardana and Elmore recently developed an interesting photocatalytic process for carbon-14 labeling with $Pd(PPh_3)_4$.[11] The intermediacy of alkyl radical was proposed, although competing direct amination of primary alkyl iodides represents a major side reaction. Odell and co-workers reported an alternative but noteworthy photoreductive route to aliphatic amides with alkyl iodides applying low pressure CO,[12] though this method was not applied for isotope labeling. Here, an acyl radical species was proposed as the key intermediate generating an acyl iodide for the ensuing acylation step. As in the previous work, competing direct alkylation of the amine proved to be a major obstacle. For carbon-11 isotope labeling, Odell and Eriksson developed a thermal mediated N-acylation of amines with alkyl iodides, generating the same radical intermediates as in the photoreductive approach (Scheme 2).[13] Whereas the chemistry proved successful, the study was confined to the synthesis of relatively simple carboxamides. Finally, Rhaman, Långström and Halldin reported a

nickel(0)-promoted aminocarbonylation of simple alkyl iodides with $^{11}CO$.[14] Key to this success was the need for air-sensitive $Ni(COD)_2$ and bathophenathroline as the ligand, and as before elaborate chemical structures were not explored.

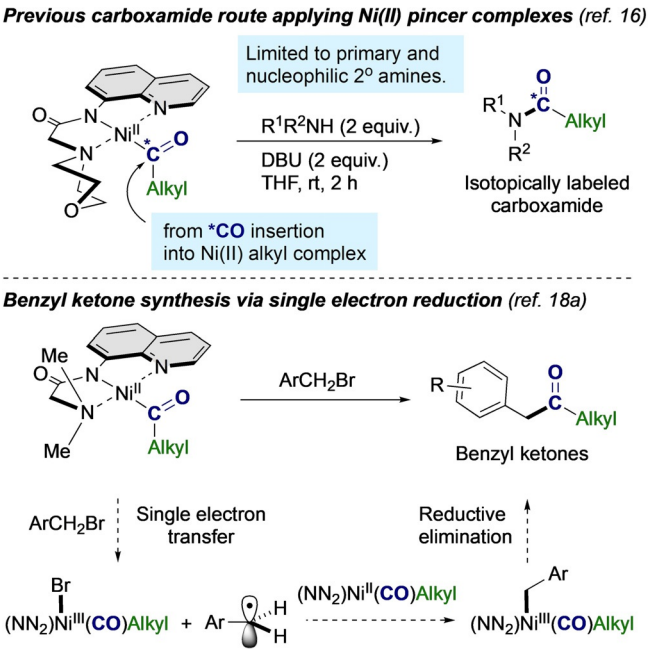
With the exception of the traditional approach to carbon isotope labeling of aliphatic carboxamides starting from strongly basic and nucleophilic organometallic reagents, all other approaches rely on the presence of the amine coupling partner in the carbonylation reaction mixture. Whereas such routes allow for direct product formation, they are nevertheless limited to carboxamides of limited complexity, and because of the very nature of the reaction conditions or necessary solvents, none seem compatible with amidation reactions for installing a carbon isotope into biopharmaceuticals. In this paper, we report on an alternative approach to carboxamide synthesis relying on the generation of isotopically labeled and reactive thioester intermediates via the carbonylative coupling of mild-basic alkyl zinc reagents[15] with disulfides (Scheme 3). Direct addition of the amine without thioester isolation provides suitable yields for a wide range of carboxamides, including the biopharmaceuticals, Victoza® (liraglutide) and Tresiba® (insulin degludec). The methodology was tested for carbon-13 labeling, but the reaction conditions were optimized and show potential for both carbon-14 and carbon-11 isotope labeling as well.




**Scheme 3.** Development of a thiocarbonylation approach applying Ni-pincer complexes for the efficient formation of carbon-labeled activated esters.

## Results and Discussion

In 2018, we reported the application of a nickel/$NN_2$ pincer complex for the $Ni^{II}$-mediated aminocarbonylation yielding aliphatic carboxamides (Scheme 4).[16] Crucial for successful amide bond formation was the presence of the morpholino group on the ligand as similar efforts with a pincer ligand bearing a dimethylamine group proved less rewarding. Although the work provided a novel approach to the target functionality, the scope of this aminocarbonylation was still relatively limited, working best with primary amines or nucleophilic secondary amines, such as morpholine or piperidine. Other amines proved demanding for carboxamide synthesis, which was attributed to a challenging $Ni^{II}/Ni^{0}$ reductive elimination step and the steric hindrance surrounding the metal center, thereby impeding attack from more encumbered amine nucleophiles. Furthermore, attempts to adapt the chemistry to carbon-11 isotope labeling were fruitless.[17] Efforts

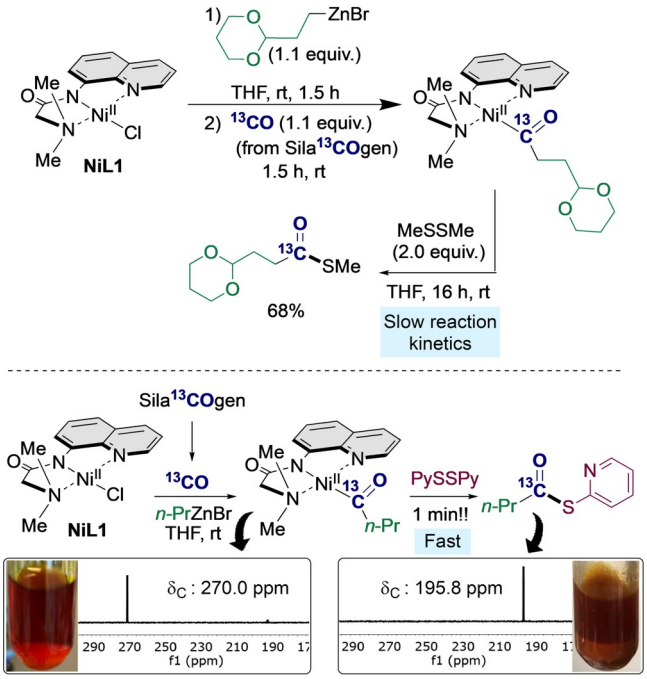



**Scheme 4.** Previous studies on the use of $(NN_2)Ni^{II}$ complexes for the synthesis of carboxamides and benzyl ketones.

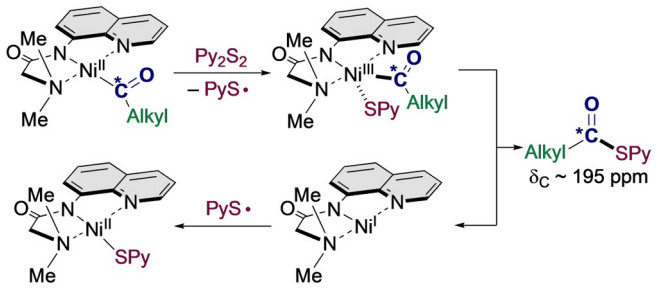
were also undertaken to generate in situ an activated ester including the *N*-hydroxysuccinimide ester or a thioester using the corresponding alcohol or thiol. However, yields were under 30% for the former, and whereas the two thiols tested provided a yield of 66 and 74% of the thioesters, considerable reaction times (2.5 h) were necessary for competition.[16]

With a desire to identify a more efficient N-acylation technology applying our Ni-chemistry and a method, which could also be adaptable to the carbon isotope labeling of structurally more sophisticated carboxamides, we turned to some of our previous results obtained for the $Ni^{II}$-catalyzed carbonylative coupling of alkyl zinc reagents with reactive alkyl halides.[18–20] In this work, we observed that simple $(NN_2)Ni^{II}$ acyl complexes were able to react rapidly with benzyl bromides and α-bromonitriles to generate benzyl ketones and β-ketonitriles, respectively. Further investigations suggested the presence of single electron transfer steps in the mechanism, and a nickel(II/III/I) catalytic cycle involving bimetallic oxidative addition (Scheme 4). Because, of the inherent ability of the $(NN_2)Ni^{II}$ acyl complex to promote single electron transfer, we speculated whether easy reducible disulfides could be combined with such $Ni^{II}$-complexes for the efficient generation of reactive thioesters, which subsequently could be used for the acylation of amines.[21] And if sufficiently quick within the minutes time scale, the chemistry could stand a chance as a viable route for carbon-11 ($t_{1/2} \approx 20$ min)[4c] labeling of pharmaceutically relevant carboxamides.

Initial efforts in this direction are depicted in Scheme 5. A transmetallation step involving the $(NN_2)Ni^{II}Cl$ (**NiL1**), bearing a dimethyl amine group, with an alkyl zinc reagent proceeded smoothly in THF at room temperature. A succeeding CO insertion step could be carried out applying our two-chamber technology in combination with the $^{13}C$-labeled carbon monoxide




**Scheme 5.** Initial studies on the reaction of $(NN_2)Ni^{II}$-acyl complexes with disulfides for the preparation of thioesters.




**Scheme 6.** Proposed mechanism for the transformation of $(NN_2)Ni^{II}$-acyl complexes to thioesters with dipyridyl disulfide via a single electron transfer pathway.

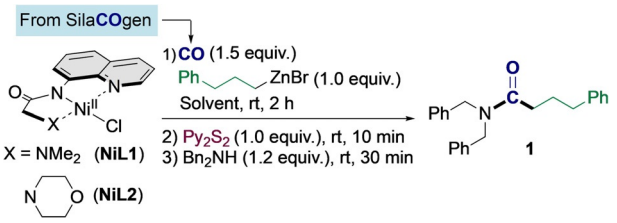
surrogate, sila$^{13}$COgen,[22] providing a stoichiometric amount of labeled gas. Room temperature reaction of the $Ni^{II}$-acyl complex with dimethyl disulfide proved successful to generate the methyl thioester. However, monitoring of the reaction progression revealed that at least 16 h were necessary for its completion. As such an alternative disulfide was tested. To our delight, the reaction of 2,2′-dipyridyl disulfide with the $Ni^{II}$-acyl complex, formed from the combined treatment of the **NiL1** with $^{13}$CO and *n*-propyl zinc bromide, showed a quantitative conversion to the thioester within one minute as observed by the visible change of the red colored solution of the $Ni^{II}$-acyl complex into a brown solution with precipitation. Furthermore, product formation was evidenced by $^{13}$C NMR spectroscopic analysis of the reaction mixture before and after the disulfide addition. This rapid and quantitative formation of the 2-pyridyl thioester from crystalline and nontoxic 2,2′-dipyridyl disulfide provides a convenient and easy route for the synthesis of an active ester. Besides serving as viable acylation reagents for amidations, the 2-pyridyl thioesters are also reactive towards a variety of carbon-centered nucleophiles and exploited in the Corey–Nicolaou macrolactonization and the Fukuyama ketone synthesis.[23]

A mechanism similar to that proposed for reaction of the $Ni^{II}$-acyl complex with activated alkyl halides is proposed and illustrated in Scheme 6.[18] Single electron transfer from nickel(II) to the disulfide results in the abstraction of a pyridylthiyl fragment forming a $Ni^{III}$(SPy)(acyl) complex and the generation of a thiyl radical. Reductive elimination of the nickel(III) intermediate subsequently leads to the desired thioester and a $Ni^{I}$-complex. The latter is a strong reductant,[24] and undoubtedly capable of reducing the disulfide as well, or combining with a thiyl radical, which in both cases will yield a $Ni^{II}$-SPy end-product. By analyzing the precipitation formed from the reaction with disulfide by $^1$H NMR spectroscopy, we observed the signals corresponding to a $Ni^{II}$-SPy compound (see Supporting Information).

With these results in hand, we speculated whether these series of intermediate reactions for thiocarbonylation and amidation could be converted into a one-pot protocol in order to improve the overall scope for accessing a wide variety of aliphatic carboxamides compared to our previous work.[16] Furthermore, if successful, such a protocol would obviate the need for inert and dry reaction conditions after the thioester has been prepared, in contrast to the use of $Ni^{II}$-acyl complexes as the direct acylation reagent. This may prove advantageous for the acylation of amine compounds that require water as a (co)solvent for solubility, for example, biomolecules including peptides and proteins. Thioesters are well known to be stable under aqueous conditions in contrast to other active esters such as acid chlorides, despite their similar reactivity.[21] The optimization of the one-pot reaction with dibenzylamine to yield *N*,*N*-dibenzyl-4-phenyl-butanamide (**1**) was initiated (Table 1), a transformation with secondary amines, which was unsuccessful in our previous carboxamide synthesis efforts.[16]

THF was initially used as the solvent to form the $Ni^{II}$-acyl complex from CO and 3-phenylpropyl zinc bromide in a two-chamber reactor with stirring for 2 h. The 2-pyridyl thioester was thereafter prepared from the direct addition of 2,2′-dipyridyl disulfide, and after 10 min, dibenzylamine was added. Amide bond formation was complete after 30 min, leading to a 78% yield of the carboxamide **1** based on $^1$H NMR analysis of the reaction mixture (entry 1). Adding triethylamine in step 3 resulted in a similar yield (entry 2), showing that the reaction is already sufficiently basic for the C–N bond forming step. In contrast to our previously reported work on carboxamide synthesis, exchanging **NiL1** for **NiL2** decreased the yield to a small extent (entry 3). Changing the solvent from THF to MeCN did not produce a significant effect on the yield (entry 4), however, the extensive precipitation formed after the addition of the disulfide now went into solution facilitating stirring of the reaction. Increasing the amount of the amine, disulfide or heating the reaction revealed small increments in the yield of carboxamide up to 87% yield (entries 5–7). On the other hand, the reaction performed in the absence of the disulfide only provided a 3% yield of product (entry 8), demonstrating its im-

**Table 1.** Optimization of the one-pot synthesis of carboxamides starting from the in situ generated $Ni^{II}$-acyl complex.[a]




| Entry | Deviations from initial conditions | Solvent | Conv. [%][b] |
|---|---|---|---|
| 1 | none | THF | 78 |
| 2 | $NEt_3$ (1.5 equiv) added to amine | THF | 75 |
| 3 | **NiL2** instead of **NiL1** | THF | 66 |
| 4 | MeCN instead of THF | MeCN | 78 |
| 5 | $Bn_2NH$ (2.0 equiv) | MeCN | 80 |
| 6 | $Py_2S_2$ (2.0 equiv) | MeCN | 87 |
| 7 | reactions run at 70 °C | MeCN | 87 |
| 8 | No $Py_2S_2$ | MeCN | 3 |
| 9 | (3-phenylpropyl)-ZnBr (1.5 equiv) | MeCN | 47 |
| 10 | reversed stoichiometry[d] | MeCN | 71 |
| 11 | reversed stoichiometry[d] | THF | [97][c] |
| 12 | reversed stoichiometry,[d] COgen[e] | THF | 100 |

[a] All reactions were set up with a two-chamber system on a 0.1 mmol scale (see Supporting Information for further details). [b] Conversion was determined by $^1$H NMR analysis using $CH_2Br_2$ as an internal standard. [c] Yield of the isolated product. [d] **NiL1** (1.5 equiv), CO (2.0 equiv), 3-phenylpropyl zinc bromide (1.5 equiv), $Py_2S_2$ (1.5 equiv), $Bn_2NH$ (1.0 equiv, limiting). Reaction time of step 3 was increased to 2 h. [e] SilaCOgen was exchanged with COgen (2.0 equiv), $PdCl_2$(COD) (5 mol%), $HBF_4$·P(*t*Bu)$_3$ (5 mol%), $Cy_2NMe$ (3.0 equiv) in THF (1.0 mL) and the reaction time for step 1 was increased to 4 h.

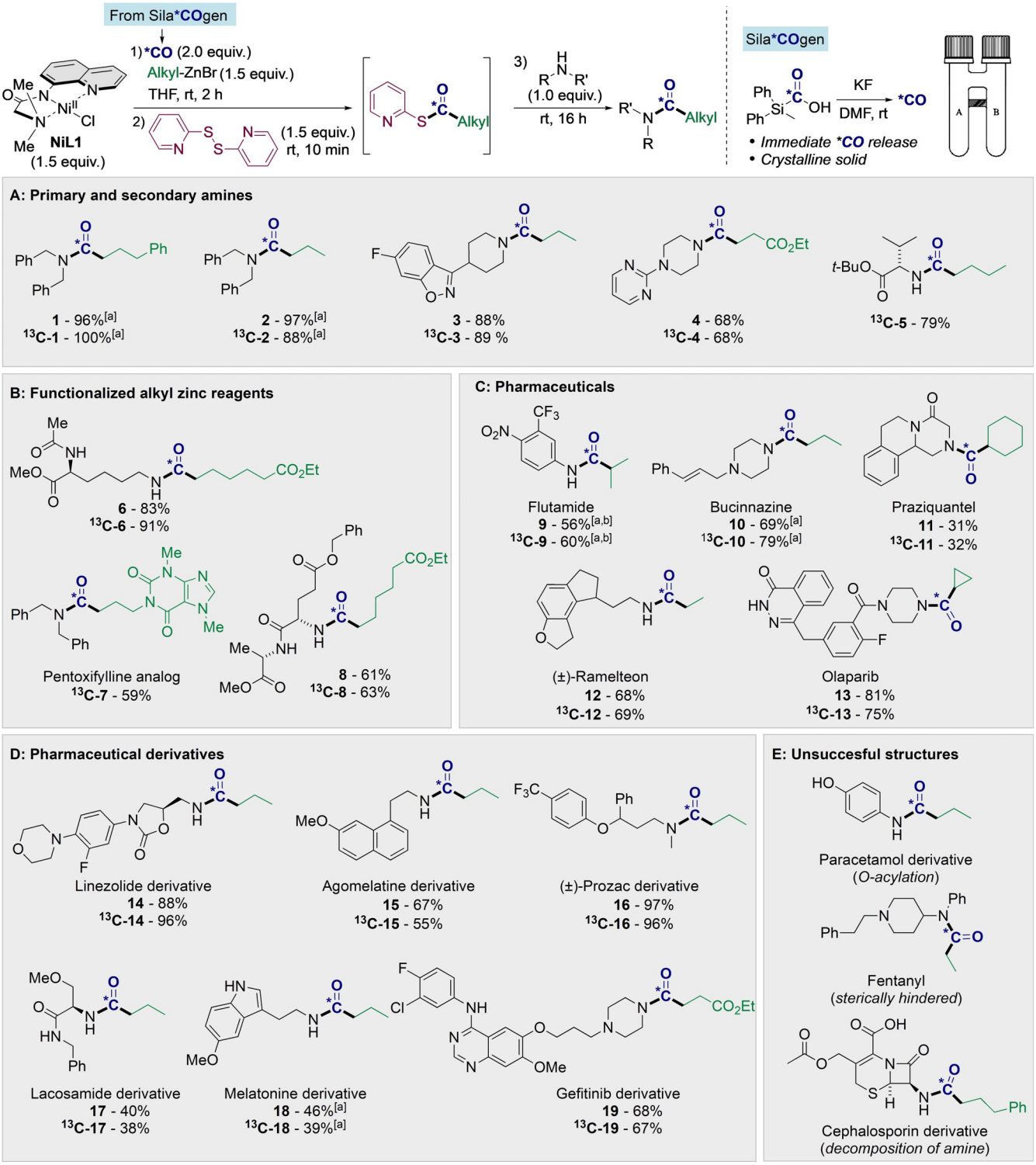
portance for this sequential transformation. Upon addition of excess alkyl zinc reagent, a deleterious side reaction was observed, giving rise to the carbonylative homodimer of the alkyl zinc reagent, namely 1,7-diphenylheptan-4-one. This also lowered the yield of the carboxamide product down to 47% (entry 9). To avoid this side reaction, titration of the alkyl zinc reagent prior to use is recommended, such that all of the reagent is consumed during the transmetallation step. Lastly, in an attempt to obtain a quantitative yield of the carboxamide, the reaction was performed with the amine as the limiting reagent. Using 1.5 equivalents of **NiL1** in step 1, the same number of equivalents for the disulfide in step 2, and a reaction time of 2 h for step 3 did not increase the yield of the reaction (entry 10). But when changing the solvent back to THF, a quantitative conversion was observed, both when using SilaCOgen and COgen as the CO-releasing molecules (entries 11 and 12).[22,25] We propose this solvent effect to be the result of a cleaner generation of the $Ni^{II}$-acyl complex as a build-up of a $^{13}$C-side product is seen when using acetonitrile (a signal at 192.8 on the $^{13}$C NMR spectrum is observed suggesting the formation of a stable Ni(CO) complex). Particularly attractive is the observation that a quantitative yield of the carboxamide can be attained with COgen as the CO source. Unlike SilaCOgen, both $^{13}$COgen and $^{14}$COgen are commercially available, opening the door for carbon-13 and carbon-14 labeling of carboxamides without the pre-synthesis of the CO releasing molecules. We have also earlier demonstrated that once carbonylation conditions with $^{13}$COgen as the CO source have been optimized, the same reaction conditions can easily be transferred to carbon-14 labeling.[26]

With the optimized conditions for the one-pot protocol in our hands, we proceeded to investigate the substrate scope of this methodology (Scheme 7). Initially, the efficiency of the protocol with both primary and secondary amines was investigated, and yields of 68–100% of the desired carboxamides **1**–**4** and the $^{13}$C-variants (**$^{13}$C-1**–**$^{13}$C-5**) were obtained as shown in section A. Some structural variance in the alkyl zinc reagents on the reaction outcome was demonstrated (section B), which include both carboxyl esters and a heterocyclic fragment derived from the pharmaceutical pentoxifylline (compounds **6**, **8**, **$^{13}$C-6**–**$^{13}$C-8**). The three compounds were obtained in good to excellent yields (59–91%). Furthermore, the synthesis of four known carboxamide-bearing pharmaceuticals, including flutamide (**9**), bucinnazine (**10**), ramelteon (**12**), olaparib (**13**), and their $^{13}$C-labeled counterparts, was achieved in good to high yields (56–81%, section C). Only in the case of praziquantel (**11** and **$^{13}$C-11**) was the yield down to 31%, a result which can be ascribed to the less efficient transmetallation step with secondary alkyl zinc reagents as previously observed.[18a] On the other hand, reverting to use of the less bulky cyclopropyl zinc bromide restored the yields as demonstrated in the case of olaparib (**13** and **$^{13}$C-13**).

Unfortunately, adaptation of this amidation protocol to acetamide synthesis proved unrewarding. Nevertheless, we have earlier reported the successful application of methyl palladium complexes as a starting point for $^{11}$C- and $^{13}$C-isotope-labeling of a variety of N-acetylated peptides.[27] On the other hand, our method proved ideal for accessing pharmaceutical derivatives with longer alkyl chains, a situation which would be problematic with Pd-mediated aminocarbonylations due to β-hydride elimination.[9] This included the synthesis of six variants of commercial pharmaceuticals, linezolide (**14**), agomelatine (**15**), prozac (**16**), lacosamide (**17**), melatonine (**18**) and gefitinib (**19**) along with their corresponding $^{13}$C-isotope analogs (section D). Interestingly, in the case of **19**, a piperazine derivative of gefitinib, this compound was shown to possess significant activity towards human cancer cell lines.[28]

For the substrate scope, certain structures proved reluctant to efficient N-acylation with our procedure (section E). O-Acylation was shown to be competitive in attempts to prepare the propyl derivative of paracetamol. In the unsuccessful case of fentanyl, we believe steric hindrance of the amine nucleophile was most likely detrimental for the N-acylation step. On the other hand, significant decomposition was observed in the attempted adaptation of our acylation chemistry with a cephalosporin derivative as monitored by $^1$H NMR spectroscopy of the crude reaction mixture.

The successful demonstration of this chemistry to carbon-13 labeling of aliphatic carboxamides holds promise for its adaptions to labeling with carbon-14 as earlier demonstrated with Pd-catalyzed aminocarbonylations to $^{14}$C-labeled benzamides and benzoic acids.[26] However, for $^{11}$C-labeling, significantly faster reaction times would be compulsory because of the

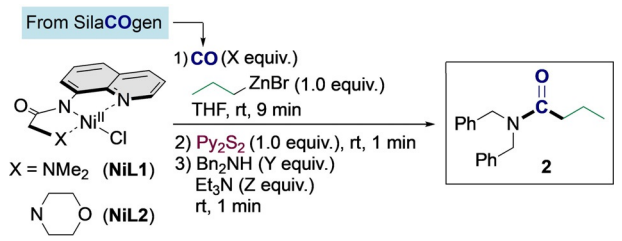



**Scheme 7.** Substrate scope for the $Ni^{II}$-mediated synthesis of aliphatic carboxamides via 2-pyridyl thioesters with and without $^{13}C$-labeling. All reactions were set up in a two-chamber system on a 0.1 mmol scale (see Supporting Information for further details). [a] The reaction time for step 3 was 2 h. [b] The aniline nucleophile (0.1 mmol, 1.0 equiv) was stirred with NaH (1.0 equiv) in THF (1.0 mL) for 2 h, and thereafter added in step 3.

short half-life of carbon-11 in the minutes range. The results revealed in Table 1 and Scheme 7 with overall reaction times of several hours would not be compatible for the introduction of this short-lived radioactive carbon isotope. Hence, a new optimization of the reaction parameters was performed and illustrated with the synthesis of carboxamide **2** to examine the yield output with shorter reaction times (Table 2).

First attempt with the nickel(II) chloride **NiL1** was performed with a total reaction time of only 11 min (entry 1). In more detail, **NiL1** was reacted with CO and *n*-PrZnBr for 9 min,

**Table 2.** Optimization studies of the $Ni^{II}$-mediated synthesis of carboxamides with short reaction times.[a]



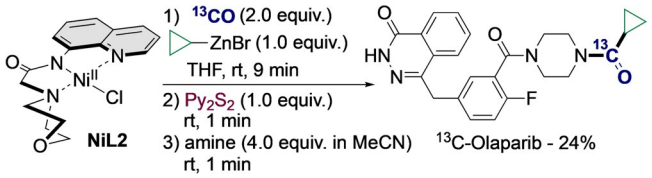

| Entry | Ni complex | CO (equiv) | $Bn_2NH$ (equiv) | $Et_3N$ (equiv) | Conv. [%][b] |
|---|---|---|---|---|---|
| 1 | **NiL1** | 1.5 | 1.2 | 1.5 | 39 |
| 2 | **NiL2** | 1.5 | 1.2 | 1.5 | 56 |
| 3 | **NiL2** | 1.5 | 2.0 | – | 56 |
| 4[c] | **NiL2** | 1.0 | 2.0 | – | 28 |
| 5 | **NiL2** | 2.0 | 2.0 | – | 65 |
| 6[d] | **NiL2** | 1.5 | 2.0 | – | 61 |
| 7[e] | **NiL2** | 1.5 | 2.0 | – | 42 |
| 8 | **NiL2** | 2.0 | 5.0 | – | 69 |

[a] All reactions were set up with a two-chamber system on a 0.1 mmol scale. $Py_2S_2$ was added from a stock solution in THF (0.4 M), and $Bn_2NH$ was added from a stock solution in MeCN (1.0 M) (see Supporting Information for further details). For entries 1 and 2, $Et_3N$ was added to the stock solutions of the amine. [b] Conversion was determined by $^1H$ NMR analysis using $CH_2Br_2$ as an internal standard. [c] **NiL2** (1.5 equiv), *n*-PrZnBr (1.5 equiv), $Py_2S_2$ (1.5 equiv). [d] CO release for 19 min. [e] All reactions run at 50 °C.

whereafter 2,2′-dipyridyl disulfide was quickly added from a stock solution in THF and allowed to react for less than a minute. Finally, *N*,*N*-dibenzylamine and triethylamine in MeCN were added and the reaction mixture was stirred for an additional minute. The efficiency of the transformation was significantly lower (39%) than for the same reaction with prolonged reaction times as shown in Scheme 7 (88–97%). This observation is likely due to the slow CO insertion that ideally requires approximately 1–2 h for a quantitative conversion as previously followed by $^1H$ NMR spectroscopy.[18a] On the other hand, resorting to complex **NiL2**, the yield pleasingly increased to 56% (entry 2). Possibly the more hemi-labile morpholino group ($pK_A$ = 8.36 in comparison with the dimethylamine, $pK_A$ = 10.6),[29] favors CO coordination to the $Ni^{II}$-alkyl complex, a prerequisite for the subsequent insertion step to generate the acyl complex.

Investigating the reaction without base, and simultaneously increasing the number of equivalents of dibenzylamine to two, led to a similar yield of 2 (entry 3), and as such further optimization studies were undertaken without the addition of triethylamine. With CO as the limiting reagent (entry 4), a lower yield of 28% was obtained. In contrast, when releasing two equivalents of CO or allowing the CO release time to occur for 19 min (entries 5 and 6), the yields increased to 65% and 61%, respectively. The modest yield obtained with a limiting amount of CO may in principle be optimized adapting different set-ups for $^{11}C$-labeling, including microfluidics systems,[30] microautoclaves,[31] or recirculation of the CO.[32]

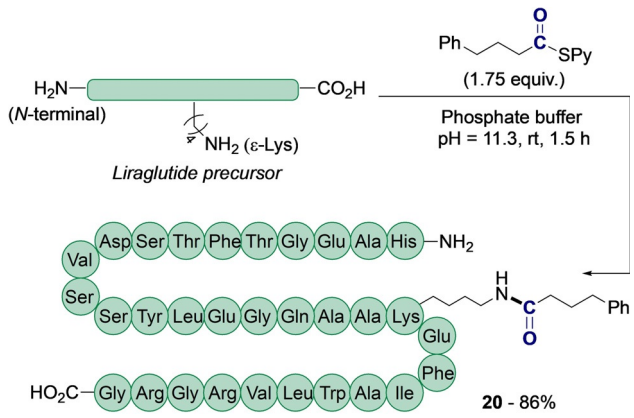
An attempt to increase the yield by heating the reaction sequence to 50 °C was not successful (entry 7). Lastly, we examined the reaction with a substantial excess of nucleophile (entry 8), a situation, in principle, which is similar to PET synthesis conditions. This modification provided a yield of 69%. Since this was only the synthesis of a rather simple carboxamide, we decided to showcase the new procedure with decreased reaction times for the synthesis of $^{13}C$-olaparib, as a proof-of-concept (Scheme 8). This carbon-13 labeled pharmaceutical could be isolated in a 24% yield after a total reaction time of just 11 min, indicating its potential application for $^{11}C$-labeling.




**Scheme 8.** Rapid carboxamide synthesis of $^{13}C$-olaparib applying the nickel(II) chloride complex **NiL2**.

Finally, we turned to the possibility of exploring this chemistry for the expedient carbon isotope labeling of biopharmaceuticals carrying an aliphatic carboxamide motif, such as liraglutide and insulin degludec, both representing antidiabetic drugs.[33] Nevertheless, for successful N-acylation to be realized with biomolecules, there are several challenging obstacles that must be dealt with. Firstly, in cases whereby more than one free amine containing residue are present in the biomolecule of interest, the N-acylation must be performed with high site selectivity for these amine-containing residues. Secondly, solubility and stability of the relevant biomolecule in a basic and possibly aqueous reaction medium may become an issue. With respect to the selective N-acylation of the liraglutide precursor, this large peptide containing 31 amino acid residues displays two reactive amines, one at the N-terminal position and the other at the ε-lysine site. Even more complicated for the synthesis of the insulin derivative, insulin degludec, three amines must be chemically distinguished, namely the N-terminal positions for the two peptide strands, $Gly^{A1}$, $Phe^{B1}$, and the terminal $Lys^{B29}$ residue. Lastly, the solubility of the isotopically labeled acylation reagent should be compatible with the solubility of the peptide, and the reagent must also be stable towards hydrolysis in the basic medium in which these acylation reactions take place.

Initially, we evaluated the use of a simple and unlabeled 2-pyridyl thioester in the reaction with the peptide precursor for liraglutide, and found a good conversion to the mono-N-acylated product **20** (Scheme 9). The peptide product was not fully characterized other than by UPLC/MS, but a high selectivity for the acylation of the ε-lysine was expected, as previously demonstrated for other acylations to the ε-acylated product under reaction conditions at high pH (10–12).[34,35] However, solubility issues were a reoccurring problem with other 2-pyridyl thioesters tested. Thus, to solve this concern, we turned to synthesize

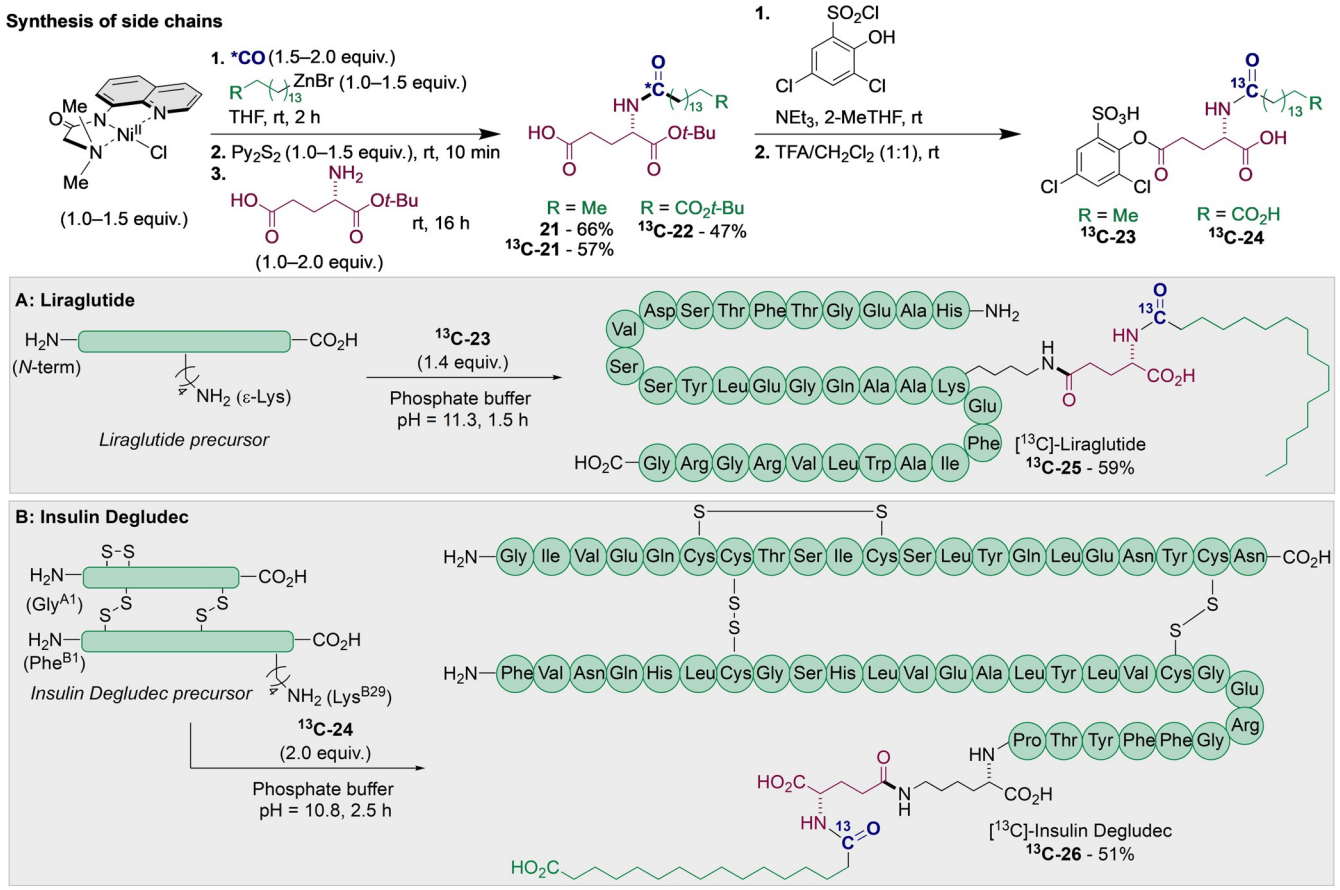



**Scheme 9.** Initial studies on the site selective N-acylation of liraglutide with an S-pyridyl thioester.

the isotopically labeled side chains of both antidiabetic drugs exploiting our one-pot carboxamide protocol as illustrated in Scheme 10. Since the reaction tolerated the presence of unprotected carboxylic acids, these side chains could subsequently be easily transformed into the phenol-based active esters **$^{13}$C-23** and **$^{13}$C-24**. Importantly, the unlabeled versions have recently been reported to selectively target the desired ε-lysine amine of the peptide precursor to liraglutide.[35] With compound **$^{13}$C-23** in hand, $^{13}$C-labeled liraglutide was isolated in a satisfactory yield of 59% after isolation by preparative HPLC (Scheme 10, section A). Furthermore, to our delight, by the same strategy, the larger biopharmaceutical insulin degludec could be prepared with a specific $^{13}$C-label on the side chain installed at the Lys$^{B29}$ residue with an isolated yield of 51% (section B). As described earlier, the selective acylation of the liraglutide and insulin degludec precursors at the ε-Lys and Lys$^{B29}$, respectively, applying 3,5-dichloro-2-hydroxy-benzenesulfonate (dchbs) esters occurs at a high pH.[34,35] Furthermore, in the case of both peptide products, the site-selective acylation was confirmed by digestion of the $^{13}$C-labeled peptides followed by MS analysis (see Supporting Information for further details).

## Conclusion

In conclusion, the one-pot synthesis of a wide selection of $^{12/13}$C-labeled aliphatic carboxamides has been achieved via the in situ generation of 2-pyridyl thioesters applying $NN_2$ nickel pincer complexes. The synthesis of known small-molecule pharmaceuticals, and their derivatives, exemplifies the attractiveness of the protocol for site selective isotope labeling, being an important prerequisite for performing early pharmacokinetic studies during drug development. Furthermore, the




**Scheme 10.** Synthesis of $^{13}$C-labeled liraglutide and $^{13}$C-labeled insulin degludec with the carbon isotope label in the side chains (see Supporting Information for further details).

possibility to successfully conduct the reaction with just 11 min, albeit with a decrease in the yield, indicates that the protocol may be viable for $^{11}$C-labeling for the potential production of PET radiotracers. Finally, the adaptation of the chemistry to $^{13}$C-labeling of biopharmaceuticals may be transferable to similar $^{14}$C-labeling of such structures, and could in future be an important tool facilitating the development of novel biopharmaceuticals.

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## Conflict of interest

Troels Skrydstrup is co-owner of SyTracks A/S, which commercializes the two-chamber system (COware®) and 13COgen.

**Keywords:** aliphatic carboxamides · aminocarbonylation · isotope labeling · nickel · thioesters

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