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PII: S0040-4039(16)30726-2
DOI: http://dx.doi.org/10.1016/j.tetlet.2016.06.057
Reference: TETL 47784

To appear in: *Tetrahedron Letters*

Received Date: 13 May 2016
Revised Date: 9 June 2016
Accepted Date: 14 June 2016

Please cite this article as: Nagao, M., Suzuki, M., Takano, Y., The practical synthesis of β-acyl glucuronides by using allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate and 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine, *Tetrahedron Letters* (2016), doi: http://dx.doi.org/10.1016/j.tetlet.2016.06.057

Tetrahedron Letters
journal homepage: www.elsevier.com

The practical synthesis of β-acyl glucuronides by using allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate and 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Acyl glucuronide
1-Chloro-*N*,*N*,2-trimethylpropenylamine
Allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate
Aryl carboxylic acid

ABSTRACT

We described the practical synthesis of β-acyl glucuronide from allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate (**5**) mediated by 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (TMCE). A wide range of bulky carboxylic acids (aryl carboxylic acids or tertiary-carbon-linked carboxylic acids) were employed to give the corresponding β-acyl glucuronate in good yields. The side products of this reaction are only *N*,*N*-dimethylisobutyramide and HCl. As the resulting acyl glucuronates show sufficient solubility to organic solvent, it can be easily purified by conventional silica gel column chromatography and/or crystallization, even in multigram quantities. The allyloxycarbonyl group and allyl group are cleanly removed by Pd(0), and thus the protocol can provide multigram quantities of β-acyl glucuronides with high purity.

Acyl glucuronides (AGs) are one of the major metabolites for drugs that contain carboxylic acid.[1] Several studies have revealed that AGs can undergo intermolecular reactions with plasma proteins, leading to covalent drug protein adducts; thus AGs have been considered to induce idiosyncratic drug toxicity.[2] As the ICH M3 guidelines show, it is preferable to evaluate the toxic profiles of reactive AGs in the early stages of drug development.

Toxicity studies require sufficient quantities of AGs with high purity thus robust and easy synthetic method that can provide them is demanded. Plausible procedures for the preparation of AGs are 1) stereoselective ester bond formation between the corresponding acid and the anomeric hydroxyl group in the protected D-glucuronic acid, and 2) chemical or enzymatic deprotection of the D-glucuronic acid moiety.[1] It was shown that the ester bond of AG is labile for acidic and/or basic conditions. Thus the reaction conditions for esterification and deprotection are both limited for the preparation of AGs.[3] However, both of those reports[1, 3] succeeded in milligram-scale preparation of AGs. To the best of our knowledge, the synthesis of multigram quantities of AGs has not been reported.

In a clinical study of KRP-105 (**1**, Figure 1), which is a highly selective peroxisome proliferator-activated receptor-γ agonist, as a candidate for anti-dyslipidemia agent,[4] it was shown that KRP-105 is readily metabolized to β-acyl glucuronide (**2**, Figure 1) in the human body. Therefore, we planned to prepare the β-acyl glucuronide of KRP-105 in multigram quantities with high purity for toxicity studies.

In this study, we investigate a synthetic method of preparing AGs in multigram quantities and describe a practical protocol for synthesizing AGs with high purity. We also report on the stereoselectivity for the acylation step in our new synthetic protocol.

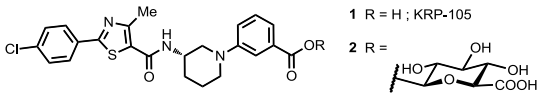

Figure 1. Structures of KRP-105 (**1**) and its β-acyl glucuronide **2**.

An established method of preparing AGs is to employ allyl D-glucuronate (**3**, Scheme 1). Under Mitsunobu reaction conditions using Ph_3P and diisopropyl azodicarboxylate (DIAD), **3** reacts with carboxylic acids to give a corresponding conjugate.[5] The resulting conjugate was transformed to AG by treatment with $Pd(Ph_3P)_4$ in conjunction with pyrrolidine. Another useful method for constructing the β-acyl bond is the condensation reaction by using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HATU).[6] Under these protocols, AGs of active pharmaceutical ingredients have been synthesized on a milligram scale.[7] Consequently, we tried to apply our substrate **1** by using the previous method.

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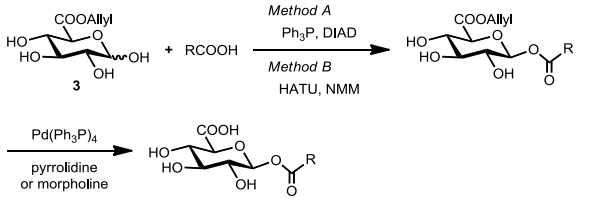
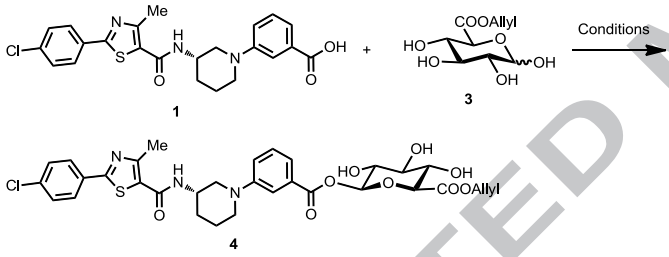


Scheme 1. General methods for the synthesis of β-acyl glucuronide.

First, we employed Mitsunobu reaction conditions for the synthesis of **2** (Scheme 2, Condition A). In the presence of Ph_3P and DIAD in THF, KRP-105 (**1**) reacts with **3** to give corresponding acyl glucuronate **4** in moderate β-selectivity (β/α = 70/30) with low conversion (31%). Severe silica gel column chromatography followed by reslurry washing in ethyl acetate gave **4** with satisfactory quality (β/α = 99.4/0.6), although the isolated yield of **4** was poor (8%).

Conversely, the condensation reaction using HATU and *N*-methylmorpholine (NMM) in acetonitrile provided excellent β/α selectivity (β/α = 93/7). However, the yield of **4** was also poor (19%) because of the low rate of conversion along with the occurrence of regioisomers such as 4-*O*-acyl glucuronate (Scheme 2, Condition B). Extensive investigations to improve the yield were unsuccessful.[8] Moreover, both the desired glucuronate **4** and its byproducts have low solubility in organic solvents and/or water. In the preparation of multigram quantities of **4**, it was assumed that purification of **4** by column chromatography or recrystallization would be problematic.



Condition A : **1** (1.0 eq.), **3** (1.0 eq), Ph_3P (1.0 eq.), DIAD (1.0 eq.), THF, rt, 15.5 h, **8%** yield
Condition B : **1** (1.0 eq.), **3** (1.0 eq), HATU (1.0 eq.), NMM (3.0 eq), MeCN, rt, 13 h, **19%** yield

Scheme 2. Synthesis of acyl conjugate **4**.

It was expected that the 2,3,4-*O*-tri-protected analog of **4** would increase solubility into organic solvents, and thus the purification of the conjugate would be easier because of its higher lipophilicity. There is also no doubt that the 2,3,4-*O*-tri-protected analog of **3** would react with acids at 1-OH to afford the desired 1-acyl conjugate exclusively. Among the protecting groups of 2,3,4-OH in **4**, the allyloxycarbonyl group seems to be more suitable for the synthesis of AGs than other protecting groups, such as acetyl, benzoyl, and benzyl, as it can be introduced in a few steps and removed under neutral conditions.[9] Therefore, we considered using allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate (**5**, Figure 2) instead of allyl D-glucuronate **3**. It has been reported that **5** reacts with aromatic isocyanate to afford the corresponding β-*O*-glycosyl carbamate.[10] However, unexpectedly, the literature contains no example of the synthesis of β-acyl glucuronides by using **5**.

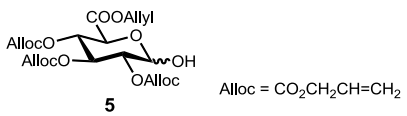
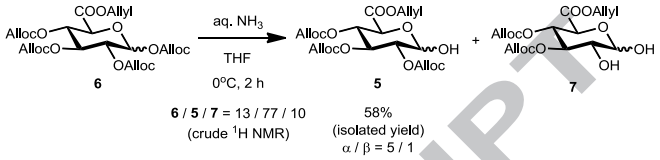


Figure 2. Structures of glucuronate **5**.

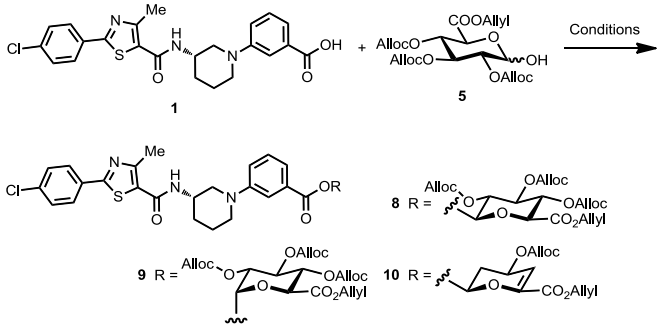
For the preparation of **5**, we examined the selective removal of the anomeric allyl carbonate in the fully protected glucuronate **6**. According to Schmidt and co-workers, bis(tributyltin) oxide is an effective reagent for selective cleavage of the anomeric allyl carbonate.[10] However, in light of its toxicity, this reagent was not suitable for the multigram preparation of **5**. It has been reported that regioselective deacylation of peracylated glycopyranoses with NH_3 in THF gave the corresponding α-1-OH sugar derivatives.[11] Therefore, we envisioned that regioselective ammonolysis would occur on anomeric allyl carbonate predominantly to give **5** selectively. As we expected, stirring **6** with ammonium hydroxide in THF at 0 ºC for 2 h afforded **5** in 58% yield (Scheme 3).



Scheme 3. Synthesis of glucuronate **5**.

Having developed a practical route to **5**, we then focused on the diastereoselective condensation of **5** with KRP-105 (**1**). Under the Mitsunobu reaction conditions, **5** reacts with **1** to give acyl glucuronate **8** in moderate β-selectivity with low yield (Table 1, entry 1). The β-selectivity diminished when the reaction was carried out using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDCI•HCl) and 1-hydroxy-7-azabenzotriazole (HOAt) (entry 2). These results required us to find another reactive intermediate. It has been reported that 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (TMCE) would form reactive carboxymethyleneiminium chloride with carboxylic acid, and that it reacts with organometallic compounds to give ketones in good yield.[12] As expected, treatment of KRP-105 (**1**) with TMCE at room temperature for 4 h followed by stirring with **5** in the presence of Et_3N gave the desired acyl conjugate **8** with high β-selectivity (β/α = 97/3) (entry 3). The yield of **8** was improved when 2 eq. of **1** and 2.5 eq. of TMCE were used (entry 4). It is conceivable that the activated iminium intermediate derived from **1** and TMCE showed not only appropriate reactivity but also the desired stereoselectivity against the anomeric β-1-OH of **5**.[13]

Table 1. Condensation reaction with KRP-105 (**1**) and **5** using TMCE.

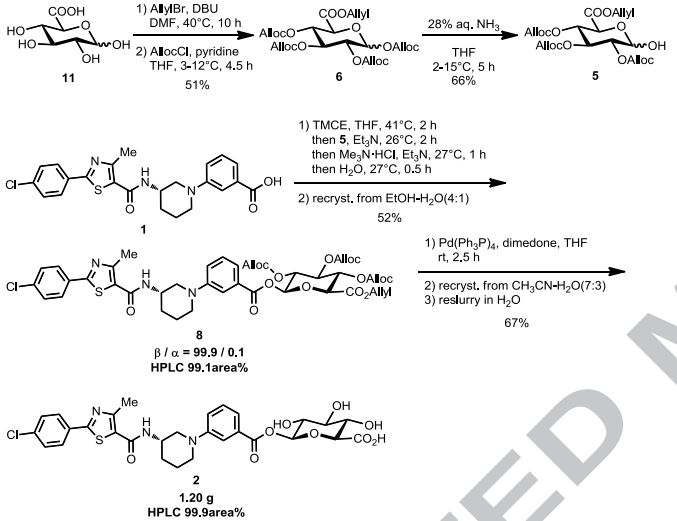


entry	conditions (equiv)	**8** / **9** / **10** [a]	yield (%) [b]
1	**1** (1.5), **5** (1.0), Ph_3P (1.5), DIAD (1.5), THF, rt,48 h	86.7 / 11.5 / 1.8	36
2	**1** (1.2), **5** (1.0), EDCI•HCl (1.2), HOAt (1.2), NMM (2.0), DMF, rt, 20 h; DMAP (0.03), rt, 53 h	50.1 / 30.1 / 19.8	55
3	**1** (1.3), TMCE (1.6), CH_2Cl_2, THF, rt, 4 h; **5** (1.0), Et_3N (1.5), CH_2Cl_2, THF, rt, 18 h	96.9 / 3.0 / 0.1	40
4	**1** (2.0), TMCE (2.5), CH_2Cl_2, THF, 40 ºC, 1 h; **5** (1.0), Et_3N (2.0), rt, 2 h	95.8 / 4.0 / 0.2	73

[a] The ratio was determined by HPLC analysis of the reaction mixture. Anomeric stereochemistry of **8** and **9** was determined by coupling constant analysis of ^1H NMR.
[b] The yield was a mixture of isolated ß-anomer (**8**), α-anomer (**9**), and 4,5-ene conjugate (**10**).

Having developed a reliable method for the β-selective acylation of KRP-105 (**1**), we applied a multigram preparation of its β-acyl glucuronide (Scheme 5). First, allyl 1,2,3,4-tetra-*O*-(allyloxycarbonyl)-D-glucuronate (**6**) was prepared in two steps

from D-glucuronic acid (**11**) according to Schmidt's procedure.[10] Regioselective ammonolysis of anomeric carbonate then furnished **5** in 66% yield. The TMCE-mediated β-selective acylation of **5** with **1** proceeded to provide the desired glucuronate **8** with high stereoselectivity (β/α = 95.5/4.5). The remaining **1** was precipitated after adding Me_3N•HCl followed by H_2O, thus it was removed easily from the reaction mixture. As glucuronate **8** has good solubility to organic solvent, multigram quantities (14.5 g) of **8** were prepared with sufficient quality (β/α = 99.9/0.1, HPLC 99.1area%) by using conventional silica gel column chromatography followed by crystallization. Removal of the allyloxycarbonyl group and allyl group of **8** was best effected with $Pd(Ph_3P)_4$ and 5,5-dimethyl-1,3-cyclohexanedione (dimedone) in THF to afford glucuronide **2**. The crude product was purified by flush column chromatography on acidic silica gel followed by crystallization to give 1.20 g of **2** in 67% yield with high purity (HPLC 99.9area%).[14]

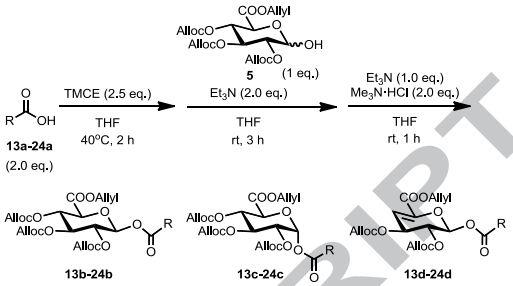


Scheme 5. Synthesis of β-acyl glucuronide **2**.

After discovering an effective acylation reaction of **5** with KRP-105 (**1**) by using TMCE, we evaluated the substrate scope for the acylation of **5** with aryl carboxylic acids. Benzoic acid (**13a**), which is a simpler substrate than KRP-105 (**1**), showed almost the same results as **1**. The TMCE-mediated acylation of **5** with **13a** gave the corresponding conjugate in 73% yield with good β-selectivity (β/α = 94/6) (Table 2, entry 1). In contrast, both the isolated yield and the β-selectivity were diminished under the Mitsunobu reaction conditions (entry 2) or using HATU as a condensation reagent (entry 3). In many cases of the use of aryl carboxylic acid, corresponding conjugates were obtained in satisfactory yield with sufficient β-selectivity by using TMCE (entries 4-9, 11, 12). It is also noteworthy that the presence of an ortho substituent, as in 2-methylbenzoic acid **18a** (entry 8), does not appreciably lower the yield. In contrast, a di-ortho substituent such as 2,6-dimethylbenzoic acid **20a** (entry 10) didn't give the coupling conjugate, because its bulkiness kept it away from the active iminium intermediate.

In the case of aliphatic carboxylic acid, the reaction proceeded well, but the β-selectivity depended on the substrate. The use of a secondary carbon directly linked to the carboxylic acid substrate, as in 4-methylphenylacetic acid **23a**, gave an α-selective coupling conjugate (entry 13). We consider that this substrate cannot show sufficient steric hindrance with β-selectivity. On the other hand, the use of a tertiary carbon directly linked to the carboxylic acid substrate, as in 2-methyl-2-phenylpropanoic acid **24a**, gave the β-selective coupling conjugate (entry 14). These results suggested that bulky carboxylic acid tends to show better β-selectivity in the condensation reaction with **5**.

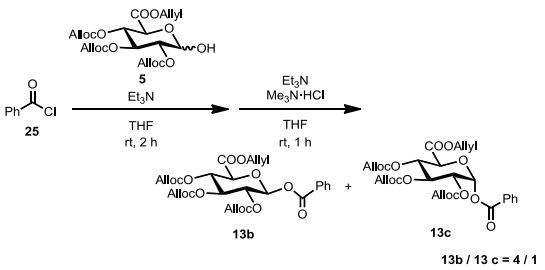
Table 2. Substrate scope for acylation of **5**.



entry	carboxylic acid: **R**	product ratio [a]	yield (%) [b]
1	**13a**: Ph	**13b** / **13c** / **13d** = 91.2 / 5.8 / 3.0	73
2[c)]		**13b** / **13c** / **13d** = 86.2 / 13.6 / 0.2	50
3[d)]		**13b** / **13c** / **13d** = 84.6 / 11.4 / 4.0	45
4	**14a**: 4-CH_3-Ph	**14b** / **14c** / **14d** = 98.4 / 1.0 / 0.6	77
5	**15a**: 4-CH_3O-Ph	**15b** / **15c** / **15d** = 84.7 / 12.1 / 3.1	58
6	**16a**: 4-Ac-Ph	**16b** / **16c** / **16d** = 75.8 / 10.9 / 13.3	78
7	**17a**: 4-Cl-Ph	**17b** / **17c** / **17d** = 86.8 / 8.4 / 4.8	79
8	**18a**: 2-CH_3-Ph	**18b** / **18c** / **18d** = 86.8 / 5.0 / 8.2	78
9	**19a**: 3-$(CH_3)_2N$-Ph	**19b** / **19c** / **19d** = 94.2 / 5.4 / 0.4	70
10	**20a**: 2,6-di-CH_3-Ph	NT	trace
11	**21a**: 4-pyridyl	**21b** / **21c** / **21d** = 72.9 / 20.1 / 7.0	85
12	**22a**: 3-thienyl	**22b** / **22c** / **22d** = 86.4 / 6.8 / 6.9	77
13	**23a**: 4-CH_3-PhCH_2	**23b** / **23c** / **23d** = 36.0 / 61.1 / 2.9	77
14	**24a**: Ph-$C(CH_3)_2$	**24b** / **24c** / **24d** = 84.5 / 14.5 / 1.0	79

[a] The product ratio was determined by HPLC analysis of the reaction mixture. Anomeric stereochemistry of the products was determined by coupling constant analysis of ^{1}H NMR. NT: not tested.
[b] The yield was a mixture of isolated in β-anomer (**b**), α-anomer (**c**), and 4,5-ene conjugate (**d**).
[c] **13a** (1.0 eq.), **5** (2.0 eq.), DIAD (2.0 eq.), Ph_3P (2.0 eq), THF, 0°C, 2 h.
[c] **13a** (1.0 eq.), **5** (1.0 eq.), HATU (1.0 eq.),NMM (2.0 eq), THF, rt, 4 h.

It has been reported that TMCE transforms carboxylic acids into the corresponding acyl chlorides via carboxymethyleneiminium chloride in CH_2Cl_2.[15] On the other hand, in a more polar solvent, such as a mixture of THF and CH_2Cl_2 (3:1), TMCE reacts with carboxylic acids to form the carboxymethyleneiminium chloride without liberation of hydrogen chloride.[12] As shown in Scheme 5, treatment of **5** with benzoyl chloride (**25**) in THF afforded corresponding acyl conjugate **13b**/**13c** with lower β-selectivity (β/α = 4/1). In contrast, adding **5** into pre-mixed TMCE and benzoic acid (**13a**) in THF gave **13b**/**13c** with high β-selectivity (Table 2, entry 1; β/α = 94/6). These results suggested that TMCE transforms benzoic acid into carboxymethyleneiminium chloride rather than acid chloride in THF (Figure 3). As its steric hindrance, the iminium intermediate reacted to the β-anomeric OH in **5**, which is more reactive than the α-OH,[13] to give β-acyl conjugate **13b** predominantly. Therefore, we consider TMCE to be an effective activating reagent for the β-selective acylation.



Scheme 5. Reaction of **5** with benzoyl chloride (**25**).

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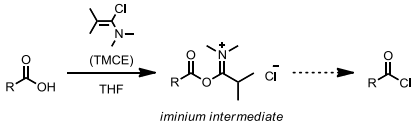

Figure 3. Proposed reaction species.

In conclusion, we demonstrated a novel method for the chemical synthesis of AGs by using TMCE-mediated β-selective acylation of allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate (**5**). It is conceivable that the activated iminium intermediate derived from carboxylic acid and TMCE showed not only appropriate reactivity but also the desired stereoselectivity against the anomeric β-1-OH of **5**. As the acyl glucuronate conjugate protected with an allyloxycarbonyl group and an allyl group show sufficient solubility to organic solvent, it can be easily purified by conventional silica gel column chromatography or crystallization, even in multigram quantities. Consequently, AGs that are difficult to synthesize in large quantities by using existing methods could be prepared with high purity. We believe this protocol should be applicable to the preparation of various AGs that possess fragile functional groups and/or low solubility.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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The practical synthesis of β-acyl glucuronides by using allyl 2,3,4-tri-(*O*-allyloxycarbonyl)-D-glucuronate and 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine

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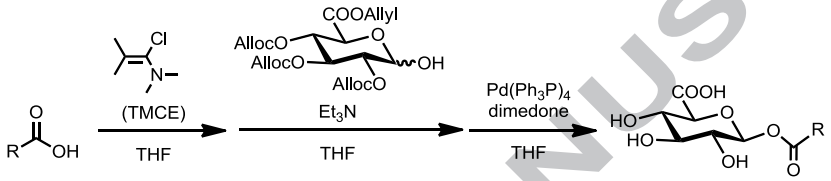

Highlights

- A practical method for the synthesis of β-acyl glucuronides is developed.
- 1-chloro-*N*,*N*,2-trimethyl-1-propylamine is a good reagent for the condensation of aryl carboxylic acids with D-glucuronate.
- Multigram quantities of β-acyl glucuronide could be prepared with high purity.

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