N,N′-Carbonyldiimidazole-Mediated DBU-Catalyzed One-Pot Synthesis of Urea-Tethered Glycosyl Amino Acids and Glycoconjugates

V. V. Sureshbabu,* Basavaprabhu

Peptide Research Laboratory, Department of Studies in Chemistry, Central College Campus, Dr. B. R. Ambedkar Veedhi, Bangalore University, Bangalore, 560 001, India
E-mail: hariccb@hotmail.com; E-mail: sureshbabuvommina@rediffmail.com; E-mail: hariccb@gmail.com
Received 8 November 2010

Abstract: An efficient, mild, simple, and alternative one-pot protocol for the synthesis of urea-tethered glycosyl amino acids mediated by *N,N′*-carbonyldiimidazole employing DBU as a catalyst is described. This protocol is also extended for the synthesis of urea-tethered disaccharides and oligosaccharides.

Key words: urea-tethered glycoconjugates, *N,N′*-carbonyldiimidazole, DBU, base catalysis

Protein glycosylation[1] is a ubiquitous process and is the most complex protein modification.[2] Generally, glycosylation involves either the ω-hydroxy group of serine[3] or carboxamide functionality of asparagine.[4] Glycopeptide mimetics involving *O*- and *N*-glycosyl linkages have been replaced by a variety of C–C, C–S, and other heteroatom bonds as well as heterocycles.[5] The urea functionality is one of the most important surrogates for the natural *N*-glycosyl moiety. This group is of paramount importance in the synthesis of a library of neoglycoconjugates which are of biological and pharmaceutical significance.[6] Approaches for the construction of urea-tethered neoglycoconjugates are relatively rare. The known strategies involve either the reaction of a glycosyl amine or amino sugar with isocyanates[7] or their synthetic equivalents such as carbamates[8] and trichloroacetimidates.[9] Other methods include addition of H_2O to the glycosyl carbodiimides[10] or by the reaction of sugar isocyanates with amines.[11,12] One-pot protocols have also been reported wherein the in situ generated sugar isocyanate will react with an appropriate amine.[13] The use of protected glycosyl isocyanates is widespread. Glycosyl isocyanates have been prepared through various protocols employing a plethora of reagents and conditions which inherently suffer from certain limitations.[12] A method involving the oxidation of glycosyl isocyanide is a multistep protocol. Acylation of amines with acid chlorides[14] is one of the most important classes of functionalization reaction used in the preparation of ureas but the carbamoyl chlorides require phosgene for their synthesis, and their relative instability does not render them suitable for long-term storage.

In order to circumvent the inconvenience associated with the preparation and handling of reactive glycosyl isocyanates, and to avoid the use of carbamoyl chlorides, our group recently demonstrated the synthesis of urea-tethered glycopeptides employing Deoxofluor/$TMSN_3$[15a] and thioureido-glycopeptides using Bt-CS-Bt.[15b] In a continuation of our interest in the area of glycopeptidomimetics, we have sought an alternative protocol for the synthesis of urea-tethered neoglycoconjugates employing *N,N′*-carbonyldiimidazole (CDI).

CDI[16] was introduced as a coupling agent in peptide synthesis and is widely used for amide bond formation. It is also used in the synthesis of glycosides and their derivatives on solid phase and in reactions involving transfer of a carbonyl group, imidazole group, and coupling between different functional groups under various conditions.

In an initial study, 2,3,4,6-tetra-*O*-acetyl-β-D-glucosamine, prepared according to the literature, was treated with valine methyl ester in anhydrous CH_2Cl_2, in the presence of CDI and *N*-methylmorpholine (NMM) at 0 °C. The reaction was found to be sluggish and yielded only 20% of the desired product after 24 hours. This is possibly due to the fact that the intermediate carbamoyl imidazole is much less reactive towards nucleophilic attack and requires activation as the carbamoylimidazolium salt[17] to effect nucleophilic substitution.

In order to improve the protocol, various bases compatible with CDI such as triethylamine (Et_3N), diisopropylethylamine (DIPEA), 4,4-dimethylaminopyridine (DMAP), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were used (Table 1). Among them, DBU[18] afforded appreciable rate enhancement with good yields of the desired product within three hours under mild reaction conditions.

Table 1 Screening of Various Bases for the Synthesis of Urea-Tethered Glycoconjugates

Base	Time (h)	Temp	Yield (%)
Et_3N	12	0 °C to r.t.	18
NMM	12	0 °C to 50 °C	20
DIPEA	6	0 °C to r.t.	45
DMAP	4	0 °C to r.t.	40
DBU	3	0 °C	88

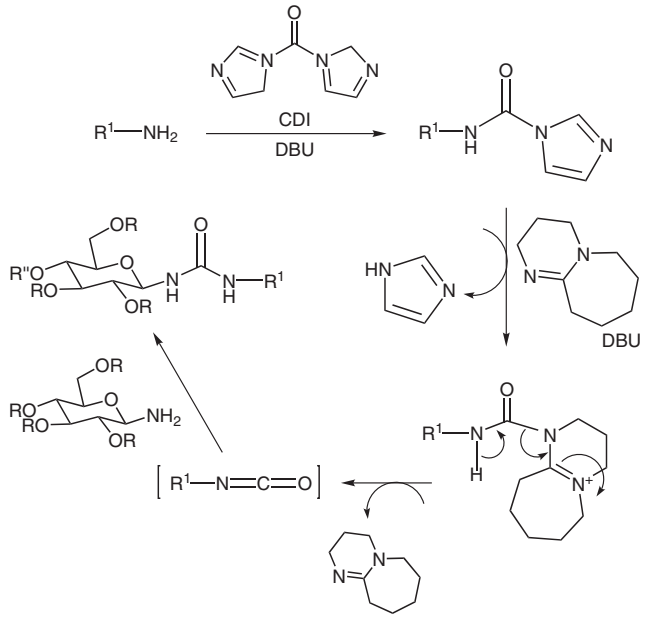
We presume that, in the first step of the reaction, DBU acts as a non-nucleophilic base, thereby assisting an

SYNLETT 2011, No. 8, pp 1160–1164
Advanced online publication: 18.04.2011
DOI: 10.1055/s-0030-1259958; Art ID: D29910ST

amine to form the carbamoyl imidazole. In the subsequent step, DBU behaves as nucleophilic catalyst where it adds to the carbamoyl imidazole intermediate with the release of imidazole byproduct.[18] The carbamoyl–DBU-linked intermediate then generates an isocyanate that subsequently reacts with the incoming sugar amine resulting in the formation of the urea (Scheme 1).

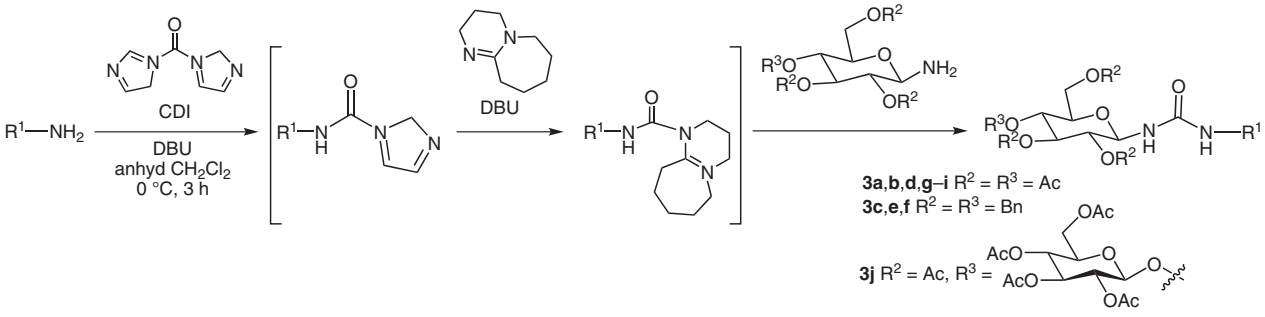
During the course of reaction, an aliquot of reaction mixture was taken and analyzed by IR spectroscopy and was found to exhibit an absorption peak at ν_{max} = 1670–1685 cm^{-1} which corresponds to the carbamoyl imidazole. During the second addition of DBU prior treatment with sugar amine, as monitored through IR, indicated the disappearance of the peak at ν_{max} = 1670 cm^{-1} with the appearance of another strong absorption peak at ν_{max} = 2246 cm^{-1}, confirming the intermediacy of the isocyanate.

Finally, IR analysis of the product showed two absorption peaks at ν_{max} = 1650 cm^{-1} and 1560 cm^{-1}, which are characteristic of the urea functionality. The structure of the final product was confirmed through ^1H NMR and ^{13}C NMR analyses. The same protocol was extended to urea-tethered pseudo-disaccharides and oligosaccharides which possess diverse biological as well as pharmaceutical applications.[19] The mild reaction conditions employed in the above-mentioned protocol (Scheme 2) were found to be epimerization-free as evidenced by ^1H NMR and HPLC analyses.[20]



Scheme 1 Possible mechanism proposed for the synthesis of urea-tethered glycoconjugates mediated by CDI under DBU base catalysis

The protocol worked well with the secondary amine of proline methyl ester (**1c**), the ω-amine of lysine (**1f**), 2,3-Dap ester (**1d**), 2,4-Dab ester (**1e**), and amines of mono- and disaccharides **1g–j** to yield the corresponding urea-tethered glycoconjugates in excellent yields (Table 2).



Scheme 2 Synthesis of urea-tethered glycoconjugates

Table 2 List of Urea-Tethered Glycoconjugates[21]

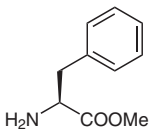
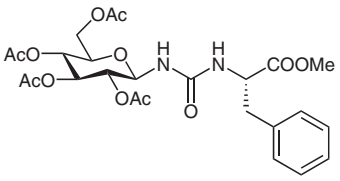
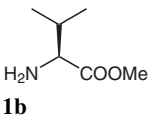
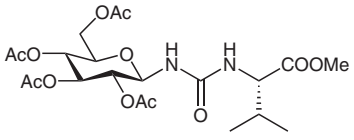
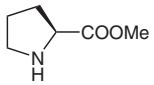
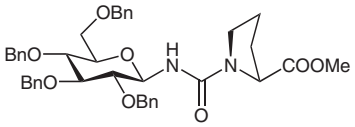
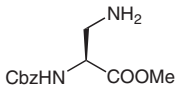
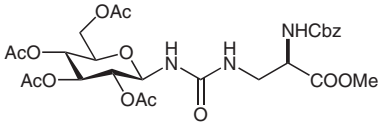
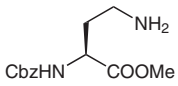
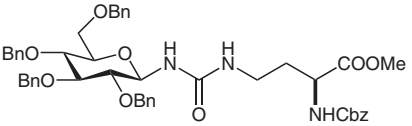
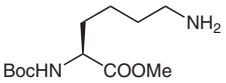
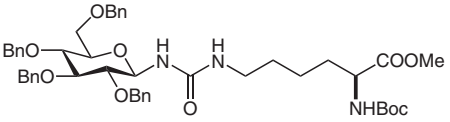
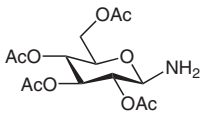
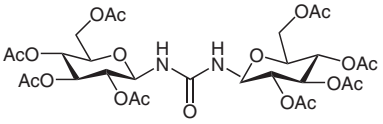
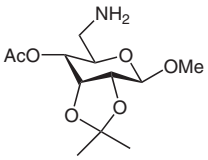
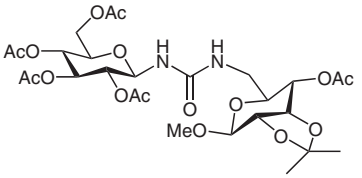
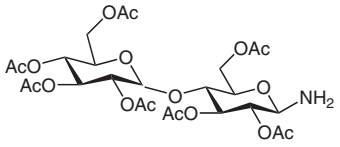
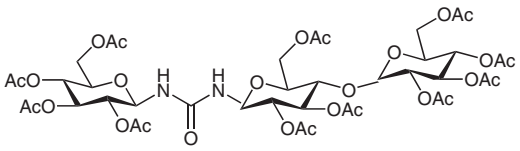
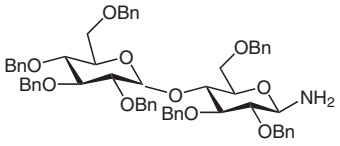
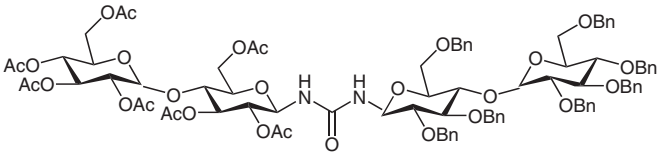
Entry	Amine	Urea-tethered glycoconjugate	Yield (%)
1	 **1a**	 **3a**	92
2	 **1b**	 **3b**	88

Table 2 List of Urea-Tethered Glycoconjugates[21] (continued)

Entry	Amine	Urea-tethered glycoconjugate	Yield (%)
3	 **1c**	 **3c**	75
4	 **1d**	 **3d**	82
5	 **1e**	 **3e**	73
6	 **1f**	 **3f**	75
7	 **1g**	 **3g**	86
8	 **1h**	 **3h**	77
9	 **1i**	 **3i**	70
10	 **1j**	 **3j**	66

In conclusion, we have developed an efficient, mild, and alternative one-pot protocol for the synthesis of urea-tethered glycoconjugates mediated by CDI in the presence of DBU as catalyst. This circumvents the isolation of reactive glycosyl isocyanates and affords high purity products in excellent yields under mild conditions.

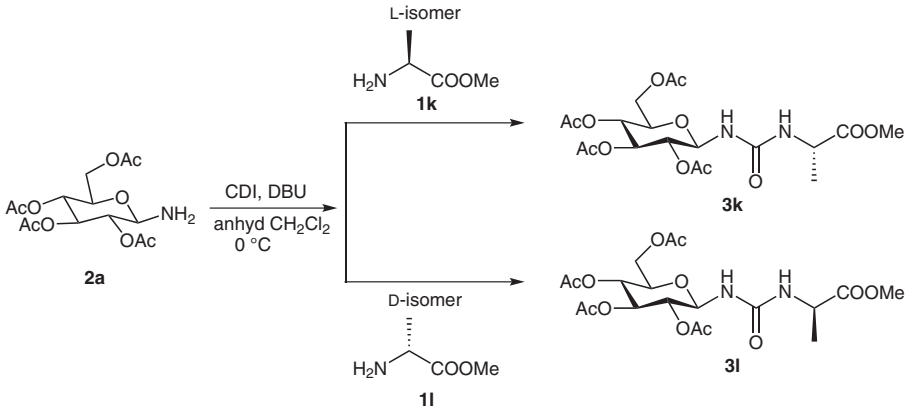
Acknowledgment

We thank the Department of Science and Technology (DST) (Grant No. SR/S1/OC-26/2008) New Delhi, India for financial support.

References and Notes

(1) Varki, A. *Glycobiology* **1993**, *3*, 97.
(2) (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (b) Selvador, L. A.; Eloffson, M.; Kihlberg, J. *Tetrahedron* **1995**, *51*, 5643. (c) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317. (d) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579.
(3) Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294.
(4) Marcaurelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384.
(5) (a) Cassarco, M. R.; Brown, R. T. *J. Org. Chem.* **2003**, *68*, 8853. (b) McDonald, F. E.; Danishefsky, S. J. *J. Org. Chem.* **1992**, *57*, 7001. (c) Khorlin, A. Y.; Zurabyan, S. E.; Macharadze, R. G. *Carbohydr. Res.* **1980**, *85*, 201. (d) Gustafson, G. L.; Gander, J. E. *Methods Enzymol.* **1984**, *107*, 172. (e) Kuijpers, B. H. M.; Groothuys, S.; Keereweer, A.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; Delft, F. L. V.; Rutjes, F. P. J. T. *Org. Lett.* **2004**, *6*, 3123. (f) *Synthesis of Peptides and Peptidomimetics (Houben-Weyl)*, Vol. E22c; Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Eds.; Thieme: Stuttgart, **2003**. (g) Cho, C. Y.; Youngquist, R. S.; Paikoff, S. J.; Beresini, M. H.; Hebert, A. R.; Berleau, L. T.; Liu, C. W.; Wemmer, D. E.; Keough, T.; Schultz, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 7706.
(6) (a) Choi, K. H.; Hamilton, A. D. *Coord. Chem. Rev.* **2003**, *240*, 101. (b) Castellano, R. K.; Nuckolls, C.; Rebek, J. Jr. *J. Am. Chem. Soc.* **1999**, *121*, 11156. (c) Cho, Y. L.; Rudkevich, D. M.; Shivanyuk, A.; Rissanen, K.; Rebek, J. Jr. *Chem. Eur. J.* **2000**, *6*, 3788.
(7) (a) Avalos, M.; Babiano, R.; Cintas, P.; Jimenez, J.; Palacios, J. C.; Valencia, C. *Tetrahedron* **1993**, *49*, 2655. (b) Avalos, M.; Babiano, R.; Cintas, P.; Jimenez, J.; Palacios, J. C.; Valencia, C. *Tetrahedron* **1993**, *49*, 2676. (c) Maya, I.; Lopez, O.; Maza, S.; Fernandez-Bolanos, J. G.; Fuentes, J. *Tetrahedron Lett.* **2003**, *44*, 8539.
(8) Nishiyama, T.; Ichikawa, Y.; Isobe, M. *Synlett* **2003**, 47.
(9) Park, N. H.; Nguyen, H. M. *Org. Lett.* **2009**, *11*, 2433.
(10) (a) Kovacs, J.; Pinter, I.; Messmer, A.; Toth, G.; Duddek, H. *Carbohydr. Res.* **1987**, *166*, 101. (b) Diaz, V. M.; Ortiz, C.; Fuentes, J.; Garcia, J. M. *Carbohydr. Res.* **1987**, *166*, 161.
(11) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *Synlett* **2000**, 1253.
(12) (a) Ichikawa, Y.; Matsukawa, Y.; Nishiyama, T.; Isobe, M. *Eur. J. Org. Chem.* **2004**, *69*, 586. (b) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *J. Org. Chem.* **2001**, *66*, 4200. (c) Ukita, C.; Hamada, A.; Yoshida, M. *Chem. Pharm. Bull.* **1964**, *12*, 454. (d) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1914**, *47*, 1377. (e) Johnson, T. B.; Bergmann, W. *J. Am. Chem. Soc.* **1932**, *54*, 3360. (f) Bannister, B. *J. Antibiot.* **1972**, *25*, 377. (g) Pinter, I.; Kovacs, J.; Toth, G. *Carbohydr. Res.* **1995**, *273*, 99.
(13) (a) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203. (b) Sawada, D.; Sasayama, S.; Takahashi, H.; Ikegami, S. *Tetrahedron Lett.* **2006**, *47*, 7219.
(14) (a) Dolle, R. E. *J. Comb. Chem.* **2001**, *3*, 477. (b) Dolle, R. E. *J. Comb. Chem.* **2000**, *2*, 383.
(15) (a) Hemantha, H. P.; Chennakrishnareddy, G.; Vishwanatha, T. M.; Sureshbabu, V. V. *Synlett* **2009**, 407. (b) Sureshbabu, V. V.; Chennakrishnareddy, G.; Hemantha, H. P. *Synlett* **2010**, 715.
(16) (a) Sharma, R. K. *Synlett* **2007**, 3073; and references cited therein. (b) Zang, X.; Rodrigues, J.; Evans, L.; Hinkle, B.; Ballantyne, L.; Pena, M. *J. Org. Chem.* **1997**, *62*, 6420. (c) Fustero, S.; Torre, M. G.; Sanz-Cervera, J. F.; Arellano, C. R.; Piera, J.; Simn, A. *Org. Lett.* **2002**, 4. 3651. (d) Figueiredo, R. M.; Fröhlich, R.; Christmann, M. *J. Org. Chem.* **2006**, *71*, 4147. (e) Weber, A. L. *Orig. Life Evol. Biosph.* **2005**, *35*, 421. (f) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776. (g) Dubé, P.; Fine Nathel, N. F.; Vetelino, M.; Couturier, M.; Aboussafy, C. L.; Pichette, S.; Jorgensen, M. L.; Hardink, M. *Org. Lett.* **2009**, *11*, 5622. (h) Opatz, T.; Kallus, C.; Wunberg, T.; Kunz, H. *Tetrahedron* **2004**, *60*, 8613. (i) Ford, M. J.; Ley, S. V. *Synlett* **1990**, 255.
(17) Oakenfull, D. G.; Salvesen, K.; Jencks, W. P. *J. Am. Chem. Soc.* **1971**, *93*, 188.
(18) Larrivee-Aboussafy, C.; Jones, B. P.; Price, K. E.; Hardink, M. A.; McLaughlin, R. W.; Lillie, B. M.; Hawkins, J. M.; Vaidyanathan, R. *Org. Lett.* **2010**, *12*, 324.
(19) Dobashi, K.; Nagaoka, K.; Watanabe, Y.; Nishida, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1985**, 1166.

(20) The epimerization study was carried out by ^{1}H NMR and HPLC analyses of the glycosyl ureas **3k** and **3l** synthesized by the protocol described below.
Glucosamine (**2a**),was converted into two epimeric glycosyl ureas **3k** and **3l** as outlined in Scheme 3 by coupling separately with L-Ala-OMe (**1k**) and D-Ala-OMe (**1l**), respectively. An equimolar mixture of these two epimers was obtained by coupling with the racemic mixture of L- and D-Ala-OMe. The ^{1}H NMR spectrum of **3k** and **3l** contained distinct methyl group doublets at δ = 1.52, 1.54 ppm and δ = 1.55, 1.58 ppm, respectively; whereas the epimeric mixture showed CH_3 group signals at δ = 1.51, 1.54 ppm and δ = 1.56, 1.59 ppm corresponding to two doublets. Additionally, HPLC analysis of the pure L- and D-Ala-OMe-derived glycosyl ureas **3k** and **3l** showed single peaks at different t_R values i.e., t_R = 9.23 and t_R = 9.61 (method: gradient 0.1% TFA H_2O–MeCN; MeCN 30–100% in 30 min), respectively.



Scheme 3

(21) **General Procedure for the Preparation of 3a–f**

To a suspension of *N*,*N*′-carbonyldiimidazole (1.2 mmol) in anhydrous CH_2Cl_2 (10 mL) was added amino acid ester (1.0 mmol) and DBU (0.2 mmol), and the reaction mixture was stirred at ambient temperature for 6–7 min. The glycosyl amine (1.0 mmol) and DBU (0.3 mmol) was then added at 0 °C, and the resulting mixture was stirred for about 3 h at 0 °C. After completion of the reaction, the resulting solution was diluted with CH_2Cl_2 (10 mL) and washed with citric acid (2 × 10 mL), H_2O (2 × 10 mL), and brine (10 mL). The organic layer was dried over anhyd Na_2SO_4, filtered, and evaporated to obtain the crude product. Pure urea-tethered glycoconjugate was obtained as a solid on column chromatography eluting with EtOAc–hexane (4:6).

The procedure followed for the synthesis of **3g–j** is similar to that described above with the only difference being the use of 0.6 mmol DBU during the addition of the second amine component.

Spectroscopic Data for Compound 3a

White solid. IR (KBr): ν_{max} = 3385, 1748, 1655, 1560, 1231 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.00 (s, 3 H), 2.03 (s, 3 H), 2.05 (s, 3 H), 2.07 (s, 3 H), 3.06 (d, 2 H), 3.69 (s, 3 H), 3.77 (ddd, *J* = 10.0, 4.3, 2.1 Hz, 1 H), 4.10 (dd, *J* = 12.3, 1.8 Hz, 1 H), 4.29 (dd, *J* = 12.3, 4.6 Hz, 1 H), 4.71 (q, *J* = 7.0 Hz, 6.0 Hz, 1 H), 4.88 (t, *J* = 9.6 Hz, 1 H), 5.10 (t, *J* = 9.4 Hz, 1 H), 5.15 (t, *J* = 9.3 Hz, 1 H), 5.12–5.22 (br, 1 H), 5.33 (d, *J* = 9.6 Hz, 1 H), 5.40–5.53 (br, 1 H), 7.04–7.16 (m, 2 H), 7.22– 7.35 (m, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.0, 21.2, 38.0, 53.1, 54.5, 62.6, 69.5, 70.2, 73.2, 74.3, 81.6, 128.4, 129.1, 130.3, 136.8, 156.5, 169.8, 170.1, 171.0, 171.6, 173.8 ppm.

Spectroscopic Data for Compound 3h

White solid. IR (KBr): ν_{max} = 1669, 1558, 1229 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.31 (s, 3 H), 1.49 (s, 3 H), 2.00 (s, 3 H), 2.02 (s, 3 H), 2.06 (s, 3 H), 2.09 (s, 3 H), 2.14 (s, 3 H), 3.32 (dd, *J* = 14.6, 5.8 Hz, 1 H), 3.41 (s, 3 H), 3.52 (ddd, *J* = 15.0, 5.8, 2.6 Hz, 1 H), 3.78 (ddd, *J* = 10.8, 5.6, 2.4 Hz, 1 H), 3.86 (ddd, *J* = 10.0, 4.2, 2.2 Hz, 1 H), 4.18 (dd, *J* = 12.6, 2.0 Hz, 1 H), 4.20 (d, *J* = 5.8 Hz, 1 H), 4.24 (dd, *J* = 7.6, 5.5 Hz, 1 H), 4.35 (dd, *J* = 12.6, 4.0 Hz, 1 H), 4.90 (dd, *J* = 10.5, 7.5 Hz, 1 H), 4.93 (t, *J* = 9.5 Hz, 1 H), 4.97 (s, 1 H), 5.12 (t, *J* = 6.0 Hz, 1 H), 5.15 (t, *J* = 9.7 Hz, 1 H), 5.19 (t, *J* = 9.4 Hz, 1 H), 5.36 (t, *J* = 9.4 Hz, 1 H), 5.47 (br d, *J* = 9.5 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.9, 21.5, 21.9, 26.8, 27.9, 42.4, 57.3, 63.9, 68.6, 70.5, 72.1, 73.3, 75.8, 76.7, 77.9, 82.1, 99.6, 110.8, 158.2, 170.4, 170.9, 172.0, 173.9, 174.5 ppm.