Application of Squaric Acid Esters in Aminodeoxy Sugar Chemistry

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The reaction of dimethyl squarate with aminodeoxy sugars, aminodeoxy alditols, amino aldonic acids, and glycosyl amines under neutral conditions furnished the squaric amide esters, which were converted in more alkaline media (pH ≈8) into the asymmetric squaric diamides. The unprotected squaric acid amide esters are suitable for constructing a specific anchor (spacer, linker) function that favorably influences the water-solubility of the resulting drugs and glycoconjugates.

In the past two decades, several drug-polymers[1,2] and important glycoconjugates[3–5] were prepared by means of the reaction of biologically active compounds with squaric acid esters **1**. Tietze et al.[1] recognized the outstanding synthetic benefit of **1**, i.e. it selectively reacts with primary or secondary amines under mild conditions in the presence of carboxyl and alcoholic, or phenolic hydroxy groups. In neutral media, the products are always the squaric acid amide esters, whereas under alkaline conditions (pH >8) the corresponding symmetric diamides are produced. Thus, squarates serve as bridge molecules for connecting two amino compounds.

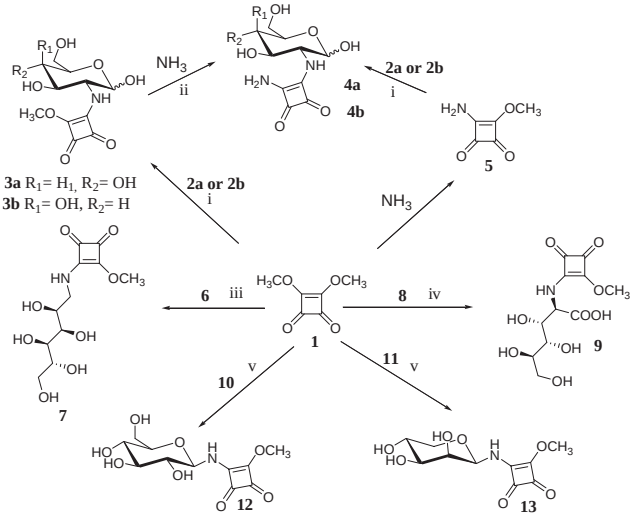
Theoretically, the squarate-coupling procedure can be extended to aminodeoxy mono- and oligosaccharides possessing a primary or a secondary amino group at any position. American authors have reported[4,5] the formation of covalent bonding between the amino group-carrying spacers of specific oligosaccharides and various seralbumins (HSA and BSA) with squaric acid esters as the first examples of utilizing squarates in the field of carbohydrates.

Recently, we applied[6,7] this simple reaction for the development of a new family of the anthracycline glycoside-type antibiotics (adriamycin, daunomycin, and carminomycin) carrying new functional groups at the secondary amino group at C-3′ of daunosamine (3-amino-2,3,6-trideoxy-L-lyxo-hexopyranose) attached via a squarate moiety.

In the present paper, we report on the extension of the squarate-derivatization to aminodeoxy sugars, aminodeoxy alditols, amino aldonic acids, and glycosyl amines by coupling with dimethyl squarate (**1**; 3,4-dimethoxy-3-cyclobutan-1,2-dion) and on the synthetic usefulness of the procedure.

The reaction of D-glucosamine hydrochloride (**2a**) and D-galactosamine hydrochloride (**2b**) with the squarate **1** furnished the monoamides **3a** and **3b**, respectively with excellent yields. Treatment of these latter compounds with a saturated solution of ammonia in methanol led to the precipitation of the D-gluco- (**4a**) and D-galacto- (**4b**) squaric diamides (Scheme 1).

Compounds **4a** and **4b** can also be synthesized by reversal of the reaction sequence. Thus, first the squaric ester monoamide **5** is prepared, which is then treated with **2a** and **2b**. However, this alternative provided the two target compounds with lower



Scheme 1. Reagents and conditions: (i) Refs. 1 and 2; (ii) MeOH saturated with NH_3, rt, 14 h; (iii) Titrisol buffer (pH 7)–MeOH, rt, 18 h; (iv) DMF–H_2O (1:1), rt, 48 h, column chromatography: Kieselgel 60, MeOH; (v) MeOH–Et_3N (pH 7), rt, 24 h, column chromatography: Kieselgel 60, CH_2Cl_2–MeOH (8:2).

yields—due to the diminished reactivity of the resulting vinyl amide ester structure.

The reaction of **1** with 1-amino-1-deoxy-D-glucitol (**6**) afforded the monoamide ester **7** with a moderate yield. At the same time, a quite sluggish reaction of D-glucosaminic acid (**8**), possessing a zwitterion structure, with **1** was observed and the product **9** could only be isolated by means of column chromatography. Although the zwitterion nature of **8** can be suspended under more basic conditions, but at pH >8 formation of the undesired diamide derivative should also be considered.

Treatment of β-D-glucopyranosylamine[8] (**10**) and β-D-lyxopyranosylamine[9] (**11**) with compound **1** furnished the methyl *N*-glycosylamino squarates **12** and **13**, respectively. These latter derivatives represent a new, specific coupling-group (anchor, spacer, and linker) in the field of the *N*-glycosides which can be directly linked to biologically active molecules under mild conditions. In such a case the linker is the 3-cyclobuten-1,2-dion skeleton.

The previously reported squaric amide esters and/or diamides possess low solubility[1] both in water and organic solvents which is a great disadvantage. In contrast, the carbohydrate–squaric acid conjugates shown in Scheme 1 are much more soluble in water.

For the utilization of this benefit of the methodology, **7** was coupled to 5-aminosalicylic acid (**14**, 5-ASA) to obtain the asymmetric squaric diamide **15** (Scheme 2). 5-ASA is used in

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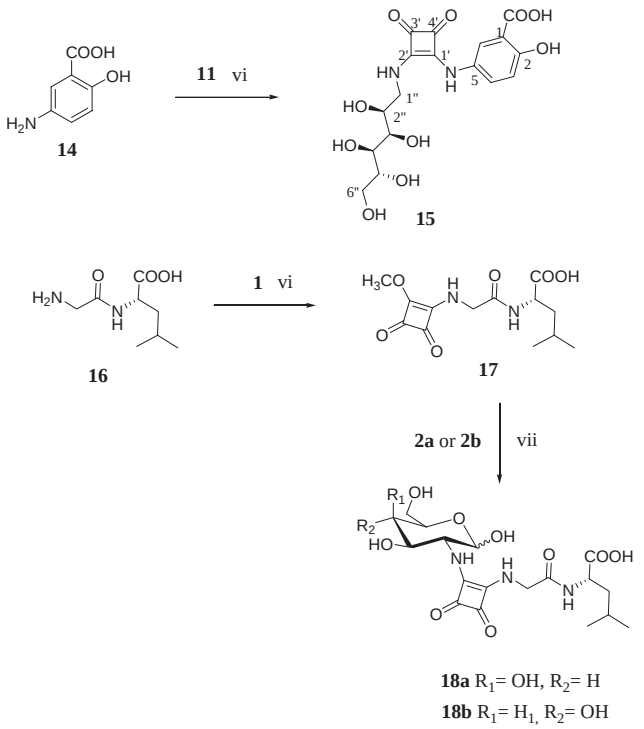

Scheme 2. Reagents and conditions: (vi) Titrisol buffer (pH 7), rt; (vii) MeOH–Et_3N (pH 7), rt, 20 h, column chromatography: Kieselgel 60, CH_2Cl_2–MeOH (8:2).

therapy for treatment of the *Chrohn*-disease, but its application is limited by the low (1 mg/mL) water-solubility. The amide **15** obtained by the above chemical modification possessed a significantly higher solubility (30 mg/mL) in water.

The reaction of **1** with L-leucylglycine (**16**) gave rise to the squaric hemi-ester dipeptide **17** also with a good yield. Treatment of **17** with **2a** and **2b** then afforded the asymmetric squaric diamides **18a** and **18b**, respectively.

The structures of all of the prepared compounds were substantiated by MALDI-TOF mass spectrometry and by ^{1}H and ^{13}C NMR measurements. As an example, characteristic spectral data for the asymmetric squaric diamide **15** {5-[3′,4′-dioxo-2′-((2″*S*,3″*R*,4″*S*,5″*R*)-2″,3″,4″,5″,6″-pentahydroxyhexylamino)-cyclobut-1′-enylamino]-2-hydroxybenzoic acid} are as follows: calculated molecular mass 412.11, found m/z $(M–H)^-$ 411.11; ^{1}H NMR data (500 MHz, D_2O, δ): 3.90–4.03, (m, 4H, H-1″$_a$, H-1″$_b$, H-6″$_a$, H-6″$_b$), 3.88–4.20 (m, 4H, H-2″–H-5″), 7.09 (s, 1H, H-3), 7.51 (s, 1H, H-4), 7.84 (s, 1H, H-6); ^{13}C NMR data (125.7 MHz, D_2O, δ): 47.53 (C-1″), 63.52 (C-6″), 72.56, 72.14, 71.96, 70.90 (C-2″–C-5″), 117.81 (C-3), 118.64 (C-1), 122.67 (C-6), 126.94 (C-4), 129.56 (C-5), 157.93 (C-1′), 165.18 (C-2′), 169.47 (C-2), 174.61 (C-7), 181.70 (C-4′), 183.77 (C-3′); $[\alpha]_D$ − 12.31 (*c*, 0.11 in water). The signals characteristic of the carbon atoms of the squaric acid residue were assigned in the ^{13}C NMR spectra of all of the corresponding derivatives. The yields and most important physico-chemical characteristics of all of the synthesized compounds are given in the Supporting Information.[10]

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This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 80th birthday.

References and Notes

1 L. F. Tietze, M. Arlt, M. Beller, K.-H. Glüsenkamp, J. Eckhardt, M. F. Rajewsky, *Chem. Ber.* **1972**, *124*, 121.
2 L. F. Tietze, C. Schröter, S. Gabins, V. Brink, A. Goerlach-Graw, H. J. Gabins, *Bioconjugate Chem.* **1991**, *2*, 148.
3 T. Rühl, M. Daghish, A. Buchynsky, K. Barche, D. Volke, K. Stembera, V. Kempin, D. Knoll, L. Hennig, M. Findeisen, R. Oehme, S. Giesa, J. Ayala, P. Welzel, *Bioorg. Med. Chem.* **2003**, *11*, 2965.
4 V. Pozsgay, E. P. Dubois, L. Pannell, *J. Org. Chem.* **1997**, *62*, 2832.
5 R. Saksena, X. Ma, P. Kovać, *Carbohydr. Res.* **2003**, *338*, 2591.
6 A. Tevyashova, F. Sztaricskai, G. Batta, P. Herczegh, A. Jeney, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4783.
7 F. Sztaricskai, A. Sum, E. Röth, I. F. Pelyvás, S. Sándor, G. Batta, P. Herczegh, J. Reményi, Z. Miklán, F. Hudecz, *J. Antibot.* **2005**, *58*, 704.
8 H. Paulsen, K. W. Pflughaupt, in *The Carbohydrates, Chemistry and Biochemistry*, 2nd ed., ed. by W. Pigman, D. Horton, Academic Press, New York, **1980**, Vol. 1B, pp. 881–927.
9 R. Brossmer, in *Methods in Carbohydrate Chemistry*, ed. by R. L. Whistler, Academic Press, New York, **1962**, Vol. 1, pp. 216–221.
10 Supporting Information is available electronically on the CSJ-Journal web site, http://www.csj.jp/journals/chem-lett/.

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