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A NEW EFFICIENT ACYLATION OF FREE GLYCOSYLAMINES

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Abstract : *Acylations of free glycosylamines 1-3 using 3-acyl(or alkoxycarbonyl)-5-methyl-1,3,4-thiadiazole-2(3H)-thiones 5 and 2-acylthio-5-methyl-1,3,4-thiadiazoles 6 as acylating reagents, provided high yields of N-acyl-glycosylamines, whereas reactions with 3-acyl-thiazolidine-2-thiones 4 caused drastic deglycosylations.*

The β-N-glycosidic linkage appears to be particularly important in various glycoconjugates [1]. Despite the large number of natural glycoconjugates including e.g. membrane glycoproteins, globulins and blood plasma proteins, it was assumed until recently that the N-glycosidic linkage resulted exclusively from the attachment of 2-acetamido-2-deoxy-β-D-glucose and 4-carboxamide group of L-asparagine. But new bonds involving D-galactose or D-glucose as sugar moities N-glycosidically linked to the amido nitrogen of a glutamine or an asparagine residue were discovered more recently in prokaryotic glycoproteins [2].

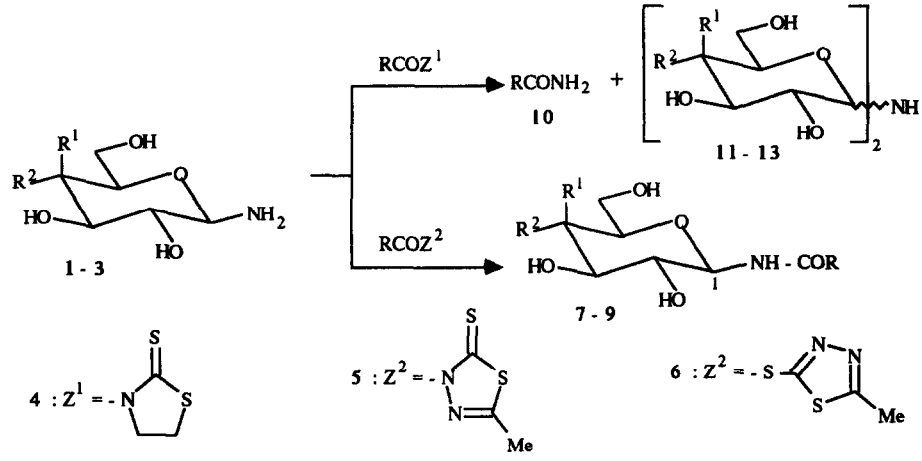
A number of model compounds were synthesized through condensation of N-protected aspartic acid α-monoesters with protected β-D-glycosylamines using DCC or EEDQ as coupling reagents [1]. The amine has so far been lengthly prepared from the fully acylated glycosyl chloride via the azide. To avoid this laborious azide route, a method based on the use of per-O-acetylglycosylisothiocyanates was also developed [3].

It is well known that unprotected glycosylamines rearrange and give browning reactions [5] and therefore they have been little used as intermediates in organic synthesis. Thus, acetolysis of free glycosylamines generally causes drastic alterations in the molecule [6].

In our work, we tried to acylate selectively β-D-glycosylamines without preliminary protection of the hydroxyl groups. Glucosylamine **1**, galactosylamine **2** and lactosylamine **3** were readily obtained when aldohexoses were treated in the cold with concentrated alcoholic solutions of ammonia [4], and the major products generally crystallized as β-anomers according to the anomeric effects. We now wish to report on acylations of compounds **1-3** by 3-acylthiazolidine-2-thiones **4**, 3-acyl(or alkoxycarbonyl)-5-methyl-1,3,4 thiadiazole-2 (3H)-thiones **5** and 2-acylthio-5 methyl-1,3,4-thiadiazoles **6**.

N-acylthiazolidine-2-thiones **4** have been previously shown by Brown [7], and later by Fujita [8] to be effective as acylating reagents for the amino group and to exhibit high chemoselectivity when reacted with aminoalcohols, including glucosamine. Surprisingly the thiones **4** [9] reacted very slowly with glycosylamines **1-3** at room temperature (table ; entry 1-2) in pyridine or DMF. When the mixture was heated at 60-70°C, decolorization occured within 4-5 hours (entry 3). The mixture provided the expected,

N-acylglycosylamines **7-9** with only 2-10 % yields, associated with a deglycosylated amide **10** (60-65 % yield) and diglycosylamines **11-13** essentially as ββ-anomers, isolated as their O-acetylated derivatives. These results are to be compared with previous works using anhydrides as acylating reagents [6,10].



1 : R^1 = H ; R^2 = OH ⟶ 7

2 : R^1 = OH ; R^2 = H ⟶ 8

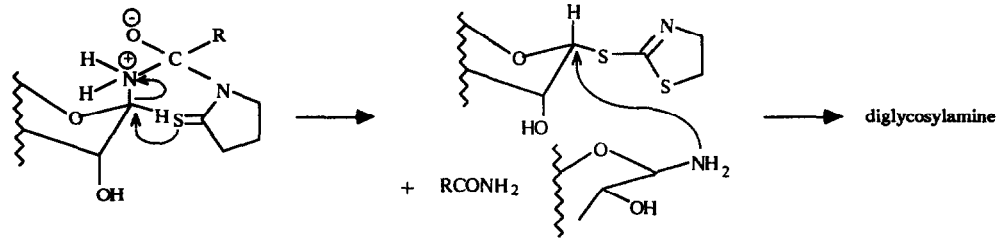
3 : R^1 = H ; R^2 = O- β- D-galactosyle ⟶ 9

R : CH_3-$(CH_2)_n$-

n	6	10	12	14	16	O_2N-C$_6$H$_4$-	Ph-CH_2O-
	a	b	c	d	e	f	g

Scheme 1

This deglycosylation is not yet well understood but can proceed through i) nucleophilic substitution at the anomeric center by the exocyclic sulphur of thiones **4** (scheme 2) that is, a von Braun-like reaction [11] or ii) a transglycosylation in which a second molecule of glycosylamine reacts with the first with the subsequent elimination of ammonia [12].



Scheme 2

In order to avoid these deglycosylations we used new acylating reagents **5-6** derived from 2-mercapto-5-methyl-1,3,4-thiadiazole [13,14]. These compounds have been previously proved to be more reactive than thiones **4** towards alcohols [15]. Moreover, we expected the exocyclic sulphur of **5** not to be very nucleophilic.

Indeed the reactions of 3-acyl-5-methyl-1,3,4-thiadiazole-2(3H)-thiones **5a-b,g** and 2-(4-nitrobenzoyl)-thio-5-methyl-1,3,4-thiadiazole **6f** with glycosylamines **1-3** in DMF at room temperature for 30 min to 1 h afforded the expected N-acylglycosylamines **7-9** without any deglycosylated products or amido-esters. Compounds **7-9** were purified by crytallisation or by column chromatography and thus obtained as crystalline white powders with yields of 65-90 % (Table).

Their structures were deduced from a combination of the elemental analysis and the spectrometric data [16]. As indicated in the table, this reaction appears to be of general utility for the synthesis of various glycosylamides and for the protection of the amino group of glycosylamines as an urethane.

Table - Syntheses of N-acylglycosylamines **7-9**

Entry	Starting sugar	Reagent	Reaction conditions	Products (yield %)
1	1	**4c**	C_5H_5N - 20 h at RT	**7c** (10)
2	1	**4d**	C_5H_5N - 72 h at RT	**7d** (5)
3	2	**4e**	DMF - 5 h at 60°C	**8e** (<5)
4	1	**5a**	DMF - 30 min at RT	**7a** (65)
5	1	**5b**	DMF - 30 min at RT	**7b** (70)
6	1	**6f**	DMF - 1 h at RT	**7f** (90)
7	2	**5b**	DMF - 30 min at RT	**8b** (65)
8	3	**5b**	DMF - 30 min at RT	**9b** (75)
9	1	**5g**	DMF - 90 min at RT	**7g** (68)

CONCLUSION

The readily available 3-acyl-5-methyl-1,3,4-thiadiazole-2(3H)-thiones **5** and 2-acylthio-5-methyl-1,3,4-thiadiazoles **6** reacted regiospecifically and without any deglycosylation with glycosylamines, thus affording N-acylglycosylamines in high yields. We are currently trying to apply this reaction to N-glycopeptide models.

Footnotes and References

1 - For recent reviews, see, H. Kunz, *Angew. Chem. Int. Ed. Engl.*, **26**, 294 (1987) ; R. Rocchi and V. Giormani, "*Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*", B. Weinstein, Ed., Dekker Pub., **7**, 35 (1983) ; H.G. Garg and R.W. Jeanloz, *Adv. Carbohyd. Chem. Biochem.*, **43**, 135 (1985).

2 - F. Wieland, *Biochimie*, **70**, 1493 (1988) ; F. Wieland, R. Heitzer and W. Schaefer, *Proc. Natl. Acad. Sci. USA*, **80**, 5470 (1983).

3 - A.Y. Khorlin, S.E. Zurabyan and R.G. Macharadze, *Carbohyd. Res.*, **85**, 201 (1980).

4 - M.C.A. Lobry de Bruyn, *Rec. Trav. Chim.*, **14**, 98 (1895) · *Chem. Ber.*, **28**, 3082 (1895) ; F. Micheel, R. Frier, E. Platte and A. Hiller, *Chem. Ber.*, **85**, 1092 (1952).

5 - H. Paulsen and K.W. Pflughaupt, *"The carbohydrates, Chem. and Biochem"*., 2nd Ed., Academic Press, **Ib**, 881 (1980).

6 - S. Hirano and R. Yamasaki, *Carbohyd. Res.*, **43**, 377 (1975) and *Agric. Biol. Chem.*, **39**, 995 (1975).

7 - E. Brown, R. Joyeau and M. Paterne, *Tetrahedron Let.*, 2575 (1977).

8 - Y. Nagao, K. Seno, K. Kawabata, T. Miyasaka, S. Takao and E. Fujita, *Tetrahedron Let.*, 841 (1980) ; Y. Nagao, K. Seno, K. Kawabata, T. Miyasaka, S. Takao and E. Fujita, *Chem. Pharm. Bull.*, **32**, 2687 (1984).

9 - D. Plusquellec, F. Roulleau, F. Bertho, M. Lefeuvre and E. Brown, *Tetrahedron*, **42**, 2457 (1986).

10 - J.A. Gordon, S. Blumberg, H. Lis and N.Sharon, *FEBS Lett.*, **24**, 193 (1972) ; G. Dupuis and B. Leclair, *Can. J. Chem.*, **60**, 2531 (1982).

11 - J.H. Cooley and E.J. Evain, *Synthesis*, 1 (1989) and references cited therein.

12 - B. Helferich and A. Mitrowsky, *Chem. Ber.*, **85**, 1 (1952) ; H.S. Isbell and H. L. Frush, *J. Org. Chem.*, **23**, 1309 (1958) ; R. Bognar and P. Nanasi, *Tetrahedron*, **14**, 175 (1961).

13 - The amides **5a-b** were prepared from acylchlorides and 2-methyl-5-mercapto-1,3,4-thiadiazole in dichloromethane in the presence of triethylamine. In the same conditions, the reaction of 2-methyl-5-mercapto-1,3,4- thiadiazole with 4-nitrobenzoylchloride afforded the thioester **6f** : (**5a** : m.p. < 20°C ; yield : 90 % ; **5b** : m.p. : 55-57°C, yield : 80 % ; **6f** : m.p. : 162-165°C ; yield : 85 %) ; The carbamate **5g** was previously described in : M. Allainmat, P. L'Haridon, L. Toupet and D. Plusquellec, *Synthesis*, 27 (1990).

14 - 2-Methyl-5-mercapto-1,3,4-thiadiazole was purchassed from Janssen Chimica.

15 - K. Baczko and D. Plusquellec, *Tetrahedron*, in press.

16 - For the purpose of comparison, we give here the spectrometric data corresponding to **7g** and **8b**.
N-benzyloxycarbonyl-β-D-glucosylamine **7g** : m.p. : 185-187°C (EtOH) ; yield : 68 % ; $[\alpha]^{20}_D$: + 3 (C = 0.01, EtOH) ; IR (HCB) ν (cm^{-1}) : 3500-2900 (OH and NH), 1700 (C = O) ; ^1H NMR (DMSO-d_6) δ (ppm/TMS) : 4.56 (d, 1H, H_1, J_{1-2} = 8.5 Hz) ; ^{13}C NMR (DMSO-d_6) δ (ppm/TMS) : 61.03 (C_6), 65.53 (CH_2O), 70.02 (C_4), 72.14 (C_2), 77.61 and 78.37 (C_5 and C_3), 82.54 (C_1), 127.96, 128.37, 136.85 (C_6H_5), 155.94 (C = O).
N-Lauroyl-β-D-galactosylamine **8b** : m.p. : 186-189°C (EtOH) ; yield : 65 % ; $[\alpha]^{20}_D$: + 25 (C = 0.01, DMF) ; IR (HCB) ν (cm^{-1}) : 3440 (OH), 3320 (NH), 1630 (amide I), 1550 (amide II). ^1H NMR (DMSO-d_6) δ (ppm/TMS) : 4.68 (d, 1H, H_1, J = 8.1 Hz) ; ^{13}C NMR (DMSO-d_6) δ (ppm/TMS) : 13.93 (CH_3), 22.14, 24.98, 28.77, 29.07, 31.35, 35.46 [($\underline{CH_2}$)$_{10}$-CH_3], 60.49 (C_6), 68.27 (C_4), 69.73 (C_2), 74.28 (C_3), 76.58 (C_5) 79.99 (C_1) 172,71 (C = O).

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