Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2333–2338

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

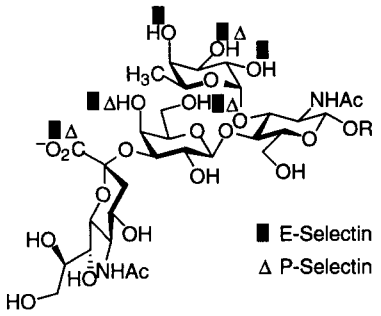
SYNTHESIS OF SIALYL LEWIS X MIMETICS USING THE UGI FOUR-COMPONENT REACTION

Chung-Ying Tsai,[a] William K. C. Park,[a] Gabriele Weitz-Schmidt,[b] Beat Ernst,[b] and Chi-Huey Wong*,[a]

[a]*Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.*
[b]*NOVARTIS Pharma AG, CH-4002 Basel, Switzerland*

Received 20 April 1998; accepted 26 May 1998

Abstract: Application of the Ugi four-component condensation to rapidly synthesize a library of glycopeptide mimics of the tetrasaccharide SLe^x as inhibitors of E- and P-selectin, and to study the effect of varied functionality in mimics on the inhibition is described.



Sialyl Lewis X

Sialyl Lewis X (SLe^x) is a tetrasaccharide found at the nonreducing end of some glycoproteins expressed on the surface of neutrophil and endothelial cells. It is also a ligand for E- and P-selectin. Because this sugar–protein interaction is a key step in inflammatory reactions and metastasis, inhibition of this interaction is considered to be a new strategy for the treatment of inflammation and metastasis.[1] Here we describe the use of Ugi four-component condensation to synthesize C-linked glycopeptides as SLe^x mimetics which are more stable than the parent structure. Three sugars of the tetrasaccharide were mimicked by peptides and we used *C*-linked D-mannose or L-fucose to replace the *O*-linked L-fucose residue. These substitutions enabled us to quickly study the effect on bonding affinity of each structural component by simply changing the acid, aldehyde, amine or isocyanide component.[2–6]

We selected compound **2** as the core structure, and by changing the R group, we studied the hydrophobic and multivalent effect.[7–11] Compound **2a–k** was prepared from aldehyde **1**,[12] glycine-PEG, methyl isocyanoacetate with various acids followed by treatment with 1 N aqueous LiOH in methanol. Using the glycine-PEG as an isolation tool, the product is poorly soluble in ether. This property is particularly beneficial for the Ugi reaction and the method is better than solid-phase synthesis since the PEG derivative is soluble in

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00422-3

protic polar solvents such as methanol or trifluoroethanol that are good for Ugi the reaction. All of the mimics were tested by the cell-free SLe^a-polymer/E-selectin assay as previously described (IC_{50} for SLe^x is 0.48 mM).[13] The results are shown in Table 1.

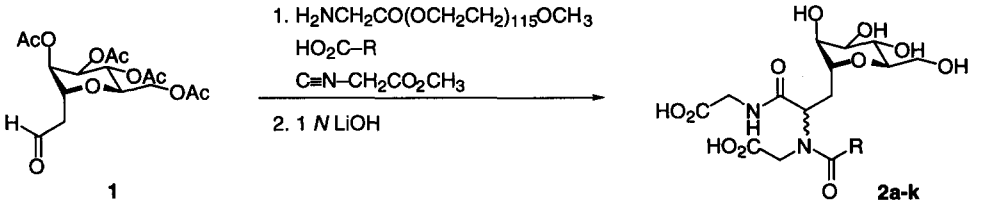
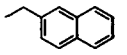
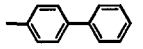
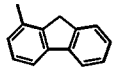
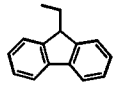


Table 1. Inhibition of E-Selectin

	R	% Inhibition at 3 mM		R	% Inhibition at 3 mM
2a	$-CH_3$	28%	**2g**		42%
2b	$-CF_3$	42%	**2h**		51%
2c	$-(CH_2)_4CH_3$	52%	**2i**		inactive
2d	$-(CH_2)_{12}CH_3$	32%	**2j**		48%
2e	$-CH=CH-CH=CHCH_3$	inactive	**2k**		inactive
2f	–Ph	38%			

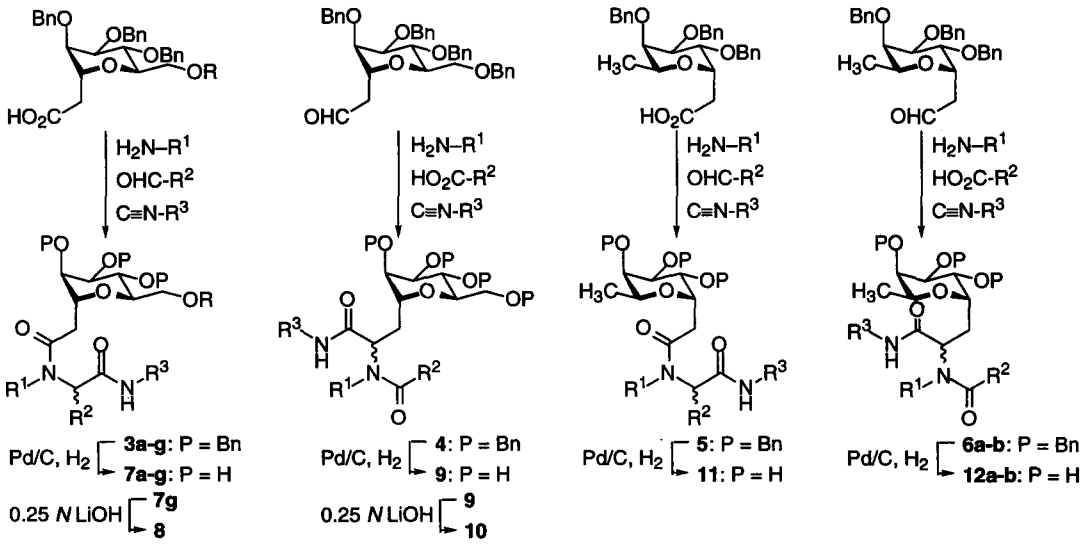
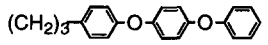
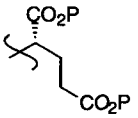
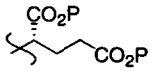
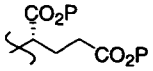
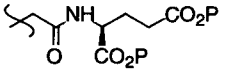
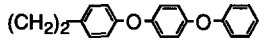
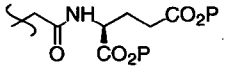
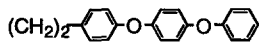
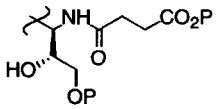


Table 2. The Ugi Reaction and Deprotection Products

R	R^1	R^2	R^3	Yield (ratio)[a]	Yield[b]
H		CH_3	CH_2CO_2Me	**3a**:65% (31:34)	**7a**:70% **7a'**:60%
$(CH_2)_4O(CH_2CH_2O)_3Et$		CH_3	CH_2CO_2Me	**3b**:53% (36:17)	**7b**:93% **7b'**:88%
		CH_3	CH_2CO_2Me	**3c**:67% (40:27)	**7c**:96% **7c'**:99%
Ph		CH_3	CH_2CO_2Me	**3d**:64% (34:30)	**7d**:79% **7d'**:70%
Ph		CH_3	$(CH_2)_2Ph$	**3e**:78% (52:26)	**7e**:84% **7e'**:76%
Ph		CH_3	$C(CH_3)_3$	**3f**:82% (53:29)	**7f**:93% **7f'**:85%
H		$(CH_2)_2CO_2P$	CH_2CO_2Me	**3g**:65% (60:<6)	**7g**:87% **7g'**:—
H		$(CH_2)_2CO_2H$	CH_2CO_2H		**8**:66%[c]

R^1	R^2	R^3	Yield (ratio)[a]	Yield[b]
	$(CH_2)_2CO_2Me$	CH_2CO_2Me	**4**:80% (20:60)	**9**:81% **9'**:98%
	$(CH_2)_2CO_2H$	CH_2CO_2H		**10**:88%[c] **10'**:79%
		$(CH_2)_2Ph$	**5**:70%	**11**:94%
		$(CH_2)_2Ph$	**6a**:71%	**12a**:78%
CH_2Ph		CH_2CO_2Me	**6b**:90%	**12b**:80%

[a]Yield of the two diastereomeric products. The number in the parenthesis is the less polar isomer to the more polar isomer ratio.
[b]The yield of the debenzylation.
[c]Isolated yield for the hydrolysis of the methyl ester.

We have also used the Ugi reaction followed by debenzylation to synthesize the new core structure mimetics **7a–g**, **9**, **11**, and **12a–b**. To investigate whether the two diastereomeric glycopeptide mimetics have different inhibition activities,[14] we separated the diastereomeric products from the Ugi reactions except for compounds **11** and **12a–b**, which can't be separated by column. Compounds **7g**, **9**, and **9′** were treated with 0.25 *N* LiOH (MeOH/H_2O, 3/1) at 4 °C to afford compound **8**, **10**, and **10′**. All the yields and ratios for the Ugi reaction and debenzylation are shown in Table 2. For comparison we also used a peptide coupling method to prepare compounds **14a–b**. Here we used two different assays to test the binding affinity: one was the cell-free

sLe^a-polyacrylamide glycoconjugate binding assay[13] and the other was the cell-free sLe^a-polylysine conjugate binding assay.[15] The IC_{50} values for E- and P-selectin are shown in Table 3.

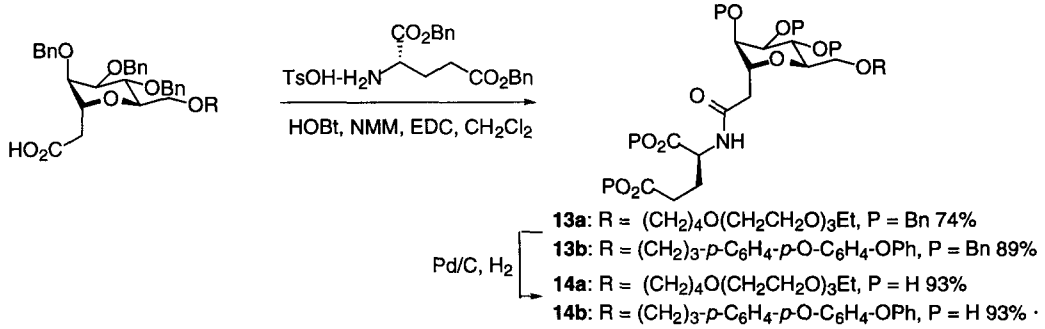


Table 3. IC_{50} Values in Selectin Inhibition[a,b]

Compound	E-selectin IC_{50} (mM)		P-selectin IC_{50} (mM)	
	Assay **A**	Assay **B**	Assay **A**	Assay **B**
SLe^x	0.48	1.09	>3.0	1.3-2.0
7a	1.00	>10	n.d.	0.007
7a'	0.36	>10	n.d.	0.118
7b	0.30	>10	>3.0	0.001
7b'	0.50	>10	>3.0	0.005
7c	0.42	3.42	1.0-3.0	0.127
7c'	0.60	3.62	1.0-3.0	0.032
7d	3.40	>2.5	n.d.	0.041
7d'	2.80	>2.5	n.d.	0.043
7e	>3.0	>2.5	n.d.	0.311
7e'	>3.0	>2.5	n.d.	0.085
7f	>3.0	>2.5	n.d.	0.087
7f'	>3.0	>2.5	n.d.	0.136
7g	3.0	>4.0	inactive	0.028
8	3.0	>4.0	inactive	0.015
9	3.0	>4.0	inactive	0.039
9'	>3.0	>4.0	inactive	0.142
10	45% inhibition at 3.0	>4.0	29% inhibition at 1	0.018
10'	45% inhibition at 3.0	>4.0	inactive	0.025
11	0.95	>2.5	n.d.	0.076
12a	0.53	>4.0	0.283	0.028
12b	0.84	n.d.	n.d.	n.d.
14a	inactive	>10	>3.0	0.020
14b	0.35	>10	0.034	0.004

[a]Assay **A** was based on cell-free sLe^a-polyacrylamide glycoconjugate binding assay,[13] assay **B** was cell-free sLe^a-polylysine binding assay.[15]
[b]All compounds are single isomer for the in vitro test except compounds **11**, **12a**, and **12b**, which are two diastereomeric mixtures. The first result is less polar isomer and the second result is more polar isomer.
[c]n.d. = not determined.

The assay results for E-selectin show that all the mimetics have similar or less activity than SLe^x. For P-selectin, however, most of the mimetics exhibit better binding affinity than SLe^x. For E-selectin, comparison of the binding affinity between two diastereomers show very little difference, but for P-selectin some diastereomeric mimetics show greater than a tenfold difference in activity. For E-selectin, introduction of the hydrophobic phenyl group at the mannose 6-position (e.g., **7d–f**) or addition of more acidic functional groups (e.g., **7g** and **8**) results in some loss of activity. Mimetics **7b** and **14b** show similar IC_{50} values with SLe^x against E-selsctin, but more active against P-selectin (IC_{50} for P-selectin **7b**: 1 μM, **7b′**: 5 μM; **14b**: 4 μM) (IC_{50} of SLe^x for P-selectin is 1.3–2.0 mM), a result consistent with previos studies.[1] Our current research is focused on the design and synthesis of SLe^x mimetics that show greater potency and increased selectivity for individual selectins.

General procedure

To a solution of 1.1 equiv aldehyde in dry methanol was dropwise added 1.3 equiv of amine in dry methanol at 0 °C under an argon atmosphere. The mixture was stirred for an additional 10 min at room temperature and followed by the sequential addition of 1.0 equiv acid in dry methanol or dry THF and 1.1 equiv isocyanide. The reaction mixture was stirred for 24–48 h at room temperature. The result solution was diluted with dichloromethane and washed with 1 *N* HCl aqueous solution followed by saturated sodium bicarbonate aqueous solution and brine. The organic layer was dried over $MgSO_4$, concentrated and purified by silica gel column chromatography (hexanes/ethyl acetate) to give two diastereomer products.

The Ugi product was mixed with 30% (w/w) of 10% Pd/C in the round bottle the vacuum removed the air and flashed with argon for three times. Vacuum removed the argon then added HOAc/THF/H_2O, 4/2/1, mixture solvent and filled with hydrogen. The reaction mixture was stirred for 18–24 h at room temperature under 1 atm hydrogen atmosphere. The result solution was filter through the Celite® removed the Pd/C, concentrated and purified by C-18 column chromatography (MeOH/H_2O) to afford a debenzylation product.

Representative physical data

7b: NMR spectra at room temperature show rotamers. ^1H NMR (400 MHz, D_2O) δ 4.60 (br s, 0.3H), 4.44–4.35 (m, 1H), 4.10–3.99 (m, 3H), 3.91–3.86 (m, 1H), 3.81–3.63 (m, 20H), 3.61–3.52 (m, 6H), 2.95 (dd, 0.7H, J = 16.2, 7.6 Hz), 2.87–2.81 (m, 0.6H), 2.74 (dd, 0.7H, J = 16.2, 5.8 Hz), 2.63–2.30 (m, 3H), 2.11–1.91 (m, 1H), 1.64–1.58 (m, 4H), 1.53 (d, 2.1H, J = 6.9 Hz), 1.50 (d, 0.9H, J = 6.8 Hz), 1.18 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100.6 MHz, D_2O, major rotamer) δ

179.96, 176.94, 175.26, 174.79, 174.19, 76.51, 76.30, 73.45, 73.15, 72.93, 72.81, 72.05, 72.02, 71.79, 71.56, 71.37, 70.05, 69.08, 59.46, 55.25, 43.77, 35.68, 34.10, 27.63, 17.52, 16.53; HRMS (FAB, M^+ + Cs) calcd for $C_{31}H_{54}N_2O_{17}Cs$ 859.2477, found 859.2501.

7b′: NMR spectra at room temperature show rotamers. ^1H NMR (400 MHz, D_2O) δ 4.92–4.86 (m, 0.7H), 4.43–4.34 (m, 1H), 4.11 (dd, 1H, J = 7.5, 3.9 Hz), 4.07 (s, 1.4H), 4.03 (d, 0.3H, J = 17.8 Hz), 3.96 (d,

0.3H, J = 17.8 Hz), 3.87–3.85 (m, 1H), 3.79–3.53 (m, 26H), 2.99–2.87 (m, 1H), 2.76–2.67 (m, 1H), 2.65–2.41 (m, 3H), 2.14–2.07 (m, 0.3H), 1.98–1.89 (m, 0.7H), 1.63–1.58 (m, 6H), 1.43 (d, 1H, J = 6.8 Hz), 1.18 (t, 3H, J = 7.1 Hz); ^{13}C NMR (100.6 MHz, D_2O, major rotamer) δ 179.91, 177.18, 177.11, 175.04, 174.04, 76.48, 76.43, 73.49, 73.15, 72.99, 72.80, 72.05, 72.02, 71.55, 71.37, 69.96, 69.07, 59.37, 55.29, 43.81, 35.15, 33.65, 28.15, 27.64, 18.36, 16.51; HRMS (FAB, $M^+ + 2Cs - H$) calcd for $C_{31}H_{53}N_2O_{17}Cs_2$ 991.1453, found 991.1429.

14b: ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 1/1) δ 7.31 (dd, 2H, J = 8.1, 7.7 Hz), 7.16 (d, 2H, J = 8.1 Hz), 7.07 (t, 1H, J = 7.4 Hz), 6.97 (d, 2H, J = 7.4 Hz), 6.96 (s, 4H), 6.91 (d, 2H, J = 8.4 Hz), 4.52 (dd, 1H, J = 8.2, 5.0 Hz), 4.36–4.29 (m, 1H), 3.80–3.70 (m, 6H), 3.53 (t, 2H, J = 6.4 Hz), 2.73–2.56 (m, 4H), 2.47–2.40 (m, 2H), 2.28–2.19 (m, 1H), 2.04–1.94 (m, 1H), 1.92–1.85 (m, 2H); ^{13}C NMR (100.6 MHz, $CD_3OD/CDCl_3$, 1/1) δ 175.95, 174.15, 172.23, 158.45, 156.30, 153.84, 153.10, 137.38, 130.28, 130.25, 123.54, 120.94, 120.56, 119.00, 118.71, 75.10, 73.87, 71.71, 71.28, 70.89, 70.59, 69.14, 52.39, 36.94, 31.88, 31.77, 30.83, 27.44; HRMS (FAB, $M^+ + 2Cs - H$) calcd for $C_{34}H_{38}NO_{12}Cs_2$ 918.0503, found 918.0541.

Acknowledgment: This research was supported by the NSF and Novartis Pharmaceuticals Ltd.

1. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833 and references cited therein.
2. Sutherlin, D. P.; Stark, T. M.; Hughes, R.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8350.
3. Keating, T. A.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8935.
4. Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 2574.
5. Armstrong, R. W.; Combs, A. P; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123.
6. Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150.
7. Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9033.
8. Wong, C.-H.; Moris-Varas, F.; Hung, S.-C.; Marron, T. G.; Lin, C.-C.; Gong, K. W.; Weitz-Schmidt, G. *J. Am. Chem. Soc.* **1997**, *119*, 8152.
9. Ramphal, J. Y.; Hiroshige, M.; Lou, B.; Gaudino, J. J.; Hayashi, M.; Chen, S. M.; Chiang, L. C.; Gaeta, F. C. A.; DeFrees, S. A. *J. Med. Chem.* **1996**, *39*, 1357.
10. DeFrees, S. A.; Phillips, L.; Guo, L.; Zalipsky, S. *J. Am. Chem. Soc.* **1996**, *118*, 6101.
11. Lin, C.-C.; Kimura, T.; Wu, S.-S.; Weitz-Schmidt, G.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2755.
12. Marron, T. G.; Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9037.
13. Weitz-Schmidt, G.; Stokmaier, D.; Scheel, G.; Nifant'ev, N. E.; Tuzikov, A. B.; Bovin, N. V. *Analytical Biochem.* **1996**, *238*, 184.
14. Tsukida, T.; Hiramatsu, Y.; Tsujishita, H.; Kiyoi, T.; Yoshida, M.; Kurokawa, K.; Moriyama, H.; Ohmoto, H.; Wada, Y.; Saito, T.; Kondo, H. *J. Med. Chem.* **1997**, *40*, 3534.
15. Thoma, G.; Magnani, J. L.; Öhrlein, R.; Ernst, B.; Schwarzenbach, F.; Duthaler, R. O. *J. Am. Chem. Soc.* **1997**, *119*, 7414.